MLSRB

MISSOURI LIFE SCIENCES RESEARCH BOARD

Spending Plan FY2010

Report to the Chair of the Senate Appropriations Committee
And Chair of the House Budget Committee

December 31, 2009

MISSOURI TECHNOLOGY CORPORATION

Missouri Life Sciences Research Board Members

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1 Vacancy

Missouri Life Sciences Research Trust Fund

History and Mission

House Bill 688 was enacted in 2003 and created the Missouri Life Sciences Research Board (MLSRB) and the Missouri Life Sciences Research Trust Fund (Trust Fund). According to the statute, "moneys in the life sciences research trust fund shall be used strategically, in cooperation with other governmental and not-for-profit private entities, to enhance the capacity of the state of Missouri's ability to perform research to better serve the health and welfare of the residents of the state of Missouri as a center of life sciences research and development by building on the success of research institutions located in Missouri, creating in and attracting to Missouri new research and development institutions, commercializing the life sciences technologies developed by such institutions, and enhancing their capacity to carry out their respective missions."

According to statute, the mission of the MLSRB and the Trust Fund is to:

- Enhance research capacity in life sciences to better serve the health and welfare of Missouri citizens;
- Promote Missouri as a center of life science research and development by building on the success of existing Missouri research institutions;
- Create and attract new research and development institutions; and
- Transform research into commercial life science technology.

At the invitation of the MLSRB, the Missouri Technology Corporation (MTC) is responsible for the administration of the Trust Fund. The MTC is a private not-for-profit corporation established pursuant to Sections 348.251 to 348.266 of the RSMo and subject to the provisions of Chapter 355, RSMo. The purpose of the MTC is to help strengthen the economy of the state through the development of science and technology; to promote the modernization of Missouri businesses by supporting the transfer of science, technology and quality improvement by providing leadership in the establishment of methods of technology application, technology commercialization and technology development.

Key responsibilities performed by the MTC on behalf of the MLSRB include drafting the request for proposals (RFPs) for the research and commercialization projects, scientific peer review services, and the centers for excellence. While the MLSRB retains all decision-making authority, MTC plays an important role in ensuring that these dollars are invested wisely by managing the grant-making and follow-up processes in a seamless, informative, and organized fashion.

Spending Plan
This spending plan is designed to provide a progress report regarding the grants funded under the Trust Fund. The Trust Fund was created in order to enhance Missouri's position as a global leader in several important sectors of the life science economy – animal science, plant science, medical devices, biomaterials and composite research, diagnostics, nanotechnology related to drug development and delivery, clinical imaging, and information technology related to human health.

The main reason for this focused approach is the inescapable fact that agriculture is Missouri's leading industry and affects every corner of the state from an economic standpoint. Another important factor, however, is that such an approach allows the state to build on tremendous strengths that already exist in plant biotechnology and animal health and nutrition. Ultimately, it is thought that placing resources where demonstrated strengths already exist will bring about the greatest results and the best return on the state's investment.

History of Funding
In 2007, House Bill 7 appropriated $13.45 million to the Trust Fund in the areas of bioenergy, plant science and animal health and nutrition. After receiving 43 proposals the LSRB awarded fourteen grants in the research and commercialization areas totaling $13.1 million.

In 2008, the General Assembly and Governor approved $21 million to the Trust Fund ($13.4 million available for new grants with the remainder going to fund ongoing projects approved by the MLSRB). The language in HB 2007, included an expansion into medical devices, biomaterials and composite research, diagnostics, nanotechnology related to drug development and delivery, clinical imaging and information technology related to human health. The MLSRB received 183 letter of intent proposals of which the MLSRB invited 66 to submit a full proposal. The MLSRB awarded eighteen grants totaling $13.1 million.

Pursuant to board policy and HB 2007, Centers for Excellence were required to focus their membership, expertise, and collaborations on all areas of research authorized by HB 2007. The following resolution was approved by the MLSRB:

> "*Recognizing that the Life Science Research Board has worked diligently to responsibly administer the monies entrusted to it by the Missouri General Assembly for fiscal year 2008, be it resolved that the Life Science Research Board strongly supports another year of funding for the Life Science Research Trust Fund and further specifies that it agrees with the recommendation to focus monies appropriated by the General Assembly exclusively on animal science, plant science, medical devices, biomaterials and composite research, nanotechnology related to drug development and delivery, clinical imaging, and information technology related to human health. Furthermore, the Life Science Research Board will not fund any human health research proposals outside of these scientific areas.*"

In 2009, the General Assembly and Governor approved $13.3 million to the Trust Fund, with $13 million available for new grants. Again, the language was specifically targeted for projects in the fields of animal science, plant science, medical devices, biomaterials and composite research, diagnostics, nanotechnology related to drug development and delivery, clinical imaging, or information technology related to human health. The MLSRB received 168 letter of intent proposals, 145 for research projects and 23 for commercialization projects. From the 168 initial proposals, the MLSRB invited 66 projects (53 research and 13 commercialization) to submit a full proposal. The MLSRB was informed in late October 2009 that the Trust Fund was included in the state spending restriction; and therefore, no grants have been awarded in Fiscal Year 2010.

The promise of the life sciences in Missouri is impressive: to provide better health for citizens and animals, to create high quality, high tech jobs across the State, and to develop an economic engine to power future growth for our communities. The General Assembly authorized the Trust Fund in 2003 to help achieve this potential.

The Trust Fund has provided economic benefits while fostering new scientific knowledge focused on improvement in healthcare, agriculture, animal health, bioenergy, and biotechnology. To assist in achieving these benefits, the MLSRB has developed thorough standards and guidelines in making and managing its awards.

The MLSRB has strived to deploy the Trust Fund to promote economic development within the State in three ways: (1) enabling new initiatives leading to the creation of jobs and additional economic activity; (2) attracting additional funding from non-state or industry resources; and (3) assisting in the commercialization of scientific innovation and the creation and development of new companies.

The awarded grants are designed to foster projects with the leveraging potential to garner additional research funding or commercialization investment. Such projects might include: major initiatives to establish new research collaboration with increased competitiveness for non-state research dollars, projects to enable institutions to recruit prominent and renowned researchers or to acquire new equipment, both of which would have the potential to pull down additional funding, and increase capacity for additional research and economic growth. Furthermore, the Trust Fund grants provide funding to projects where federal and industry funding is more difficult, especially in the cases where a successful innovation could lead to a major opportunity for future funding. Some grants provide "gap funding" for projects, bridging the divide between academic research discoveries and commercialization of products through the development of proof-of-concept activities or to develop a commercial prototype.

Below is the process designed to maximize the State's returns from its investment in the Trust Fund:

- Annual grant competitions that describe clearly and concisely the types of outcomes that are expected from funded proposals.

- Selecting proposals most likely to meet the goals. Screening Committees, which contain a panel of regional experts, and scientific peer reviewers rank proposals against criteria and make recommendations to the MLSRB about which proposals should be funded.

- Ensuring grantee accountability. Awards will be made to grantee institutions under the terms of a contract that explicitly specifies that grant funding can only be used for the purposes outlined in their proposal and are subject to reporting requirements. Furthermore, staff have met with grant recipients to ensure that funding is being spent in a prudent manner and monitored the progress of funded grants. Awardees are required to report the progress of their research against the goals stated in their proposal and account for the funding they have expended.

Goals of the Life Sciences Research Trust Fund

The goal of the Trust Fund is to promote economic vitality in the State of Missouri by fostering innovative scientific research designed to improve the welfare of the State's citizens. Financial returns will leverage the dollars expended and potentially bring outside investment. Knowledge gains will enhance the reputation of Missouri as a global center of research, discovery, innovation, and commercialization. Scientific discovery will contribute to robust industry development and economic growth. Research will lead to innovations that will improve healthcare outcomes, efficiencies in delivery, and cost-effectiveness.

Research and commercialization will generate new scientific knowledge that will promote health and strengthen the State's reputation as a hub for innovation and biotechnology. These investments will enable innovative approaches to scientific problems, new collaborations among public and private institutions, and more rapid translation of discoveries from lab to market. Furthermore, this will assist in attracting new students and investigators to the region's research community.

It will do so in five important, measurable ways:

- ***Stimulate economic activity***. The Trust Fund grants will directly fund jobs and economic activity in Missouri, paying researcher salaries, supporting scientific staff, and promoting the purchase of goods and services within the state.

- ***Commercialization***. The Trust Fund grants will assist in furthering the commercialization of research discoveries, converting ideas into innovative goods and services for the marketplace, and fostering the creation and development of new companies. This commercialization process may produce invention disclosures, patent filings, intellectual property licensing, gap-funding grants, new company formation, and equity investments. Licensing of intellectual property will garner royalty income to institutions.

- ***Leveraging additional funds***. One of the most important returns on the State's investment in the Trust Fund will be its enabling role in helping institutions leverage additional research funding from non-state sources. Furthermore, additional funding may also be in the form of private investment in regards to commercialization activities that have been supported by the Trust Fund.

- ***Growing Research Capacity***. Research capacity is the volume of directed resources available to conduct research in a specific area of interest authorized by the General Assembly. Capacity can be increased in Missouri and retained, including personnel and equipment.

- ***Expanding Knowledge***. Publications in respected peer-reviewed journals and presentations to colleagues at major scientific gatherings are two common measures for the dissemination of new discoveries. To be accepted in these venues, articles and presentations must demonstrate sound science and represent a new contribution to the body of knowledge.

Research and Commercialization Missions

The following guidelines are laid forth in state statute under Sections 196.1109, RSMo:

> "All moneys appropriated by the general assembly from the life sciences research trust fund shall be appropriated to the life sciences research board to increase the capacity for quality of life sciences research at public and private not-for-profit institutions in the state of Missouri and to thereby:
>
> (1) Improve the quantity and quality of life sciences research at public and private not-for-profit institutions, including but not limited to basic research (including the discovery of new knowledge), translational research (including translating knowledge into a usable form), and clinical research (including the literal application of a therapy or intervention to determine its efficacy), including but not limited to health research in human development and aging, cancer, endocrine, cardiovascular, neurological, pulmonary, and infectious disease, and plant sciences, including but not limited to nutrition and food safety; and
>
> (2) Enhance technology transfer and technology commercialization derived from research at public and private not-for-profit institutions within the Centers for Excellence. For purposes of sections 196.1100 to 196.1130, "technology transfer and technology commercialization" includes stages of the regular business cycle occurring after research and development of a life science technology, including but not limited to reduction to practice, proof of concept, and achieving federal Food and Drug Administration, United States Department of Agriculture, or other regulatory requirements in addition to the definition in section 348.251, RSMo.
>
> Funds received by the board may be used for purposes authorized in sections 196.1100 to 196.1130 and shall be subject to the restrictions of sections 196.1100 to 196.1130, including but not limited to the costs of personnel, supplies, equipment, and renovation or construction of physical facilities; provided that in any single fiscal year no more than ten percent of the moneys appropriated shall be used for the construction of physical facilities and further provided that in any fiscal year eighty percent of the moneys shall be appropriated to build research capacity at public and private not-for-profit institutions and twenty percent of the moneys shall be appropriated for grants to public or private not-for-profit institutions to promote life science technology transfer and technology commercialization. Of the moneys appropriated to build research capacity, twenty percent of the moneys shall be appropriated to promote the development of research of tobacco-related illnesses."

Centers for Excellence

Before each grant cycle the MLSRB approves the request for proposals (RFP) to establish the Centers for Excellence (CFE).

The first step in the process is the designation of four CFE across the state of Missouri. In order to be considered for selection as a CFE it must be established within a geographical area specified in section 196.1106, RSMo, and be comprised of a consortium of public and private not-for-profit academic, research, or health care institutions or organizations that have collectively at least fifteen million dollars in annual research expenditures in the life sciences, including a collective minimum of two million dollars in basic research in life sciences.

For organizing purposes, each CFE is required to nominate a chairman and functional board of directors representative of the consortium of public and private not-for-profit academic, research, or health care institutions or organizations associated with their CFE with a focus on agriculture research and commercialization.

Each CFE for life sciences research is required to appoint a screening committee. The CFE through their screening committees, review, prioritize, and forward to the MLSRB proposed research and commercialization initiatives for consideration for funding by the board. Members of each screening committee are required to be generally familiar with the life sciences and current trends and developments with either technical or scientific expertise in the life sciences with an understanding of life sciences and with an understanding of the application of the results of life sciences research. No member of a screening committee may be employed by any public or private entity eligible to receive financial support from the Trust Fund.

The MLSRB views the regional and statewide CFE as virtual organizations, whose purpose is to think strategically about the life science research and commercialization initiatives important to their specific region, but also how these regional initiatives strengthen the state of Missouri's ability to compete on a larger regional and national scale. The CFEs were asked to develop the strongest possible proposals within their regions, looking to collaborate among other regions to enhance and strengthen the statewide base of research and development assets wherever possible.

The MLSRB designates four Centers for Excellence during each grant cycle. They included the following geographic areas:

(1) One St. Louis area center for excellence may be established within the geographical area encompassing the city of St. Louis and St. Louis, St. Charles, Jefferson, and Franklin counties. If any part of a municipality is located within any one such county and also encompasses a part of another county in this state, the entire area encompassed within the city limits of such municipality shall be a part of the geographical area of the St. Louis area center for excellence;
(2) One Kansas City area center for excellence may be established within the geographical area encompassing Jackson, Clay, Andrew, Buchanan, and Platte counties. If any part of a municipality is located within any one such county and also encompasses a part of another county in this state, the entire area encompassed within the city limits of such municipality shall be a part of the geographical area of the Kansas City area center for excellence;
(3) One Springfield center for excellence may be established within the geographical area encompassing Greene, Christian, and Webster counties;
(4) A Missouri statewide center for excellence may be established that shall encompass the institutions, agricultural research centers dedicated to the development of plant-made pharmaceuticals, and campuses within the University of Missouri system and those regions of Missouri not encompassed within another center for excellence; provided that the University of Missouri-Kansas City and the University of Missouri-St. Louis shall participate in the Centers for Excellence in their respective geographical regions.

Centers for Excellence Chairman for the FY2010 Grant Cycle

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*** Dr. Quatrano is serving as acting chairman after the appointment of Dr. Roger Beachy as director of the National Institute of Food and Agriculture.*

Research and Commercialization Projects
The second step in the process is the approval of the RFP for letters of intent and full proposals for the research and commercialization projects. The Letter of Intent RFP is emailed to University presidents, chairs of the CFE, and members of the Research Alliance of Missouri with a clear deadline. Once the Letters of Intent are received, they are sent to the respective CFE for review by the screening committees and recommendations of priority to the MLSRB. The MLSRB then determines which projects warrant an invitation for full proposal. This entire process is accomplished within a very short timeframe from June to mid-August.

For the FY2010 grant cycle the deadline for the MSLRB to receive the letter of intent proposals was July 1, 2009. The full proposal deadline was September 14, 2009.

Proposal Criteria
Evaluations of the project proposals are based on two broad criteria:

1. **Scientific and technical quality of the proposed activity.** Proposals must address an important and relevant question(s) related to the specific research area(s) of interest to the MLSRB. The proposed project must exhibit innovation, scientific rigor and originality. The following factors will be considered in determining the project proposal's scientific and technical quality:

    - Degree of Innovation.
    - Expertise and research experience of the Principal Investigator, Co-Principal Investigators and collaborating investigators.
    - Quality and degree of collaboration(s) planned by the collaborating institutions and among the individual participants in the proposed activity and how those interactions will foster more rapid and higher quality progress toward goals of the proposed activity.
    - Inventory of any specialized facilities, equipment and/or other resources required for the proposed activity indicating if they are currently available or being sought for performance of the proposed activity including location and availability of access and how the requested resources are key to the collaborative research effort in enabling both high priority research and research collaboration.
    - Appropriate management of the proposed activity.
    - Feasibility of the scope of work proposed for the period of funding.
    - Appropriateness of the proposed budget with regard to the scope of work.

2. **Potential Impact of the Proposed Activity.** Proposals must exhibit the potential to provide a significant beneficial impact(s) to furthering the research and development capacity of the State of Missouri, job creation and the general health and welfare of the citizens of the state. The following factors will be considered in determining the proposed activity's potential impact:

    - Ability of the proposed activity to leverage additional funds from non-state sources (e.g. federal, foundation and private funding in the future to further support the specific research and/or commercialization activities.
    - Ability of the proposed activity to facilitate and promote the commercialization of discoveries and innovations that arise from research and development in the state. What are the timeline, scale, and scope of the commercialization opportunities?
    - Impact of the proposed activity on the field of research.
    - Alignment of the proposed activity with the state's strategic economic development and research priorities.
    - Potential contribution to the health and quality of life of the people of Missouri in the intermediate and/or longer term.

Funds Available In Each Grant Category
Pursuant to Section 196.1109, RSMo, eighty percent of the appropriation to the Trust Fund is available for research grants and twenty percent is available for Trust Fund commercialization grants.

It should be noted that no more than 10 percent of the Trust Fund appropriation shall be used for the construction of physical facilities or "bricks and mortar."

Furthermore, a single Center for Excellence shall not receive more than 50 percent of the annual appropriation. It should also be noted that no single institution or organization shall receive in any consecutive three fiscal year period more than 40 percent of the moneys appropriated to the Trust Fund.

Scientific Peer Review Process
The third step in the process is to initiate the Scientific Peer Review process in accordance with Section 196.1112, RSMo. The MLSRB approves the Peer Review RFP to be distributed in early July.

The Scientific Peer Review RFP requests responses from organizations or individuals routinely providing scientific peer review and related consulting services for high technology sponsored research programs. The RFP provides that neither the peer review organization nor its employees, and where applicable, its parent organization nor employees thereof, have any financial interest in any current grant, nor will they have any financial interest in any new grant awarded through the Trust Fund during the term of the proposed contract.

The following qualifications were also requested:
1. Experience in advising technical grant programs sponsoring projects within the life sciences;
2. Intimate knowledge of best practices in scientific peer review;
3. Significant experience and knowledge of issues pertaining to competitive grant programs funded by state government, including conflict of interest avoidance and public rights and access to information; and
4. Experience working with independent Web-based peer review systems.

During the Fiscal Year 2008 process, LYTMOS Group, LLC, located in Lee's Summit, Missouri was selected as the scientific peer review firm. LYTMOS demonstrated considerable experience performing similar peer reviews in the states of Florida, Pennsylvania, Maryland, and Indiana, as well as in the Kansas City area.

In Fiscal Year 2009 the peer review contract was awarded to the American Association for the Advancement of Science (AAAS) in Washington, D.C. Since 1996, AAAS has assembled and led carefully tailored teams to provide expert review and programmatic guidance on over 180 projects throughout the United States.

In Fiscal Year 2010, the MLSRB again selected LYTMOS Group, LLC as the scientific peer review firm and they completed the scientific peer review in November 2009.

Grant Awards
Within the limits of available funds, awards are made to applicants whose proposals are judged most meritorious under the evaluation criteria and procedures defined by the MLSRB. The MLSRB determines which proposals will be funded, and any conditions that might pertain to the award of funds to each selected projects.

The Trust Fund awards are to be made available within three to six weeks of the date of award notification. The expectation is that projects will be initiated within three months of award notification.

Budget

The statute under Section 196.1115.3, RSMo, allows two percent of the total appropriation to be used for administrative costs of the board and the process. The following administrative budget was approved by the MLSRB in July 2009.

Life Sciences Research Board
Approved Administration Budget
FY2010

Category	Amount Approved
Peer review	**125,000**
CFE/Screening Committee	**$15,000**
DED Staff	**$31,237**
MTC Staff	**$50,851**
Board Meeting Expenses	**$2,200**
Senior Project Manager	**$22,435**
Flex (legal/travel/misc)	**$19,277**
TOTAL	**$266,000**

Life Sciences Research Board
Administration Budget and Expenditures FY2009

Category	Amount Approved	Expended – June 30, 2009
Peer review	$127,700	**$129,976.00**
CFE/Screening Committee	$13,725	**$8,850.00**
DED Staff	$12,730	**$10,487.48**
MTC Staff	$41,200	**$22,461.06**
Board Meeting Expenses	$3,270	**544.08**
Senior Project Manager*	$44,375	**$30,097.51**
Flex (legal/travel/misc)	$25,000	**$65,583.87**
TOTAL	**$268,000**	**$268,000.00**

The Senior Project Manager position was vacant from January-June 2009. The position is currently vacant.

FY2010 Life Sciences Research Trust Fund Grant Summary

Total Grant Funding Available in FY2010: $13,000,000

Research Funds Available in FY2010: $10,400,000
Commercialization Funds Available in FY2010: $ 2,600,000

Letter of Intent Proposals Received

Centers for Excellence	Requested Research Funds	# of Research Projects	Requested Commercialization Funds	# of Commerciali-zation Projects
Kansas City	$19,136,466.00	27	$1,737,000.00	3
Springfield	$7,393,189.00	13	$809,000.00	2
St. Louis	$23,283,359.10	35	$3,920,663.00	6
Statewide	$58,775,852.76	70	$6,113,824.50	12
Subtotals	**$108,588,866.86**	**145**	**$12,580,487.50**	**23**

Full Proposals Received --Research

Centers for Excellence	Requested Research Funds	# of Requested Projects
Kansas City	$5,579,163.00	9
Springfield	$2,512,312.00	6
St. Louis	$8,520,941.00	12
Statewide	$27,574,577.38	26
Subtotals	**$44,186,993.38**	**53**

Full Proposals Received --Commercialization

Centers for Excellence	Requested Commercialization Funds	# of Requested Projects
Kansas City	$1,737,000.00	3
Springfield	$809,000.00	2
St. Louis	$1,090,663.00	3
Statewide	$3,249,496.00	5
Subtotals	**$6,886,159.00**	**13**

FY2009 Life Sciences Research Trust Fund Grant Summary

Total Grant Funding Available in FY2009: $13,100,000

Research Funds Available in FY2009: $10,500,000
Commercialization Funds Available in FY2009: $ 2,600,000

Letter of Intent Proposals Received --Research

Centers for Excellence	Requested Research Funds	# of Research Projects	Requested Commercialization Funds	# of Commercialization Projects
Kansas City	$26,680,047.00	43	$3,122,950.00	5
Springfield	$7,036,309.00	15	$1,197,600.00	2
St. Louis	$11,444,758.88	13	$4,136,000.00	5
Statewide	$67,547,438.21	88	$6,968,294.75	12
Subtotals	**$112,708,553.09**	**159**	**$15,424,844.75**	**24**

Full Proposals Received --Research

Centers For Excellence	Requested Funds	# of Requested Projects	Awarded Funds	# of Projects Awarded	% of Available Funds Awarded
Kansas City	$11,134,293.00	16	$2,432,153.00	4	23%
Springfield	$4,979,981.00	8	$825,000.00	1	8%
St. Louis	$9,177,498.20	8	$3,270,302.00	4	31%
Statewide	$29,063,772.67	27	$4,063,773.00	5	38%
Subtotals	**$54,355,544.87**	**59**	**$10,547,690.00**	**14**	**100%**

Full Proposals Received --Commercialization

Centers For Excellence	Requested Funds	# of Requested Projects	Awarded Funds	# of Projects Awarded	% of Available Funds Awarded
Kansas City	$0	0	$0	0	0%
Springfield	$574,450	1	$574,450	1	22%
St. Louis	$520,000	1	$520,000	1	20%
Statewide	$3,262,739	5	$1,505,550	2	55%
Subtotals	**$4,357,189**	**7**	**$2,600,000**	**4**	**100%**

FY2009 Funding Summary - Research

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
09-1016	Acquisition of a Confocal Laser Scanning Microscope to Enhance the Research Capabilities of University of Missouri at St. Louis	Dr. Xuemin (Sam) Wang	University of Missouri-St. Louis	Equipment	$281,745
09-1018	Derivation of Induced Pluripotent Cells from the Pig	Dr. Toshihiko Ezashi	University of Missouri	Project Proposal	$180,000
09-1019	Workforce Development and Business Incubation: Animal Health and Nutrition Infrastructure in Missouri	Dr. Gary Clapp	Missouri Western State	Equipment	$285,000
09-1053	New Medical Materials, Devices, and Instrumentation at the Jordan Valley Innovation Center	Dr. Ryan Geidd	Jordan Valley Innovation Center/ Missouri State University	Centers or Institute	$825,000
09-1055	Pseudospark Pulsed Plasma X-ray Generation for Portable Medical Devices	Dr. Joshua Rovey	Missouri University of Science & Tech	Project	$164,268
09-1065	Informatics Research Core Facility	Dr. Mark McIntosh	University of Missouri	Project	$1,302,217
09-1076	St. Louis Institute for Nanomedicine	Dr. Samuel Wickline	Washington University	Centers or Institute	$1,500,000
09-1078	Computational simulation of canine biomechanically induced unicompartmental osteoarthritis: a concurrent multiscale approach	Dr. Trent Guess	University of Missouri-Kansas City	Project	$556,957
09-1101	UMKC Center of Excellence in Mineralized Tissues	Dr. Lynda Bonewald	University of Missouri-Kansas City	Centers or Institute	$1,050,196
09-1105	Acquisition of Metabolomics Platform for Metabolic Engineering	Dr. Leslie Hicks	Danforth Plant Science Center	Equipment	$894,993
09-1106	Drought Simulators Critical to Translational Research in Plant Science	Dr. Felix Fritschi	University of Missouri	Equipment	$1,558,125
09-1117	Advanced Cardiovascular Stent incorporated with Nitric Oxide Delivery System	Dr. Chi Lee	University of Missouri-Kansas City	Project	$540,000
09-1128	Targeting Plasminogen Activator Inhibitor-1 to Inhibit Restenosis	Dr. William Fay	University of Missouri	Project	$815,625
09-1148	Optimization of Camelina as a nonfood production platform of value-added biotechnology products.	Dr. Eliot Herman	Danforth Plant Science Center	Centers or Institute	$593,564
				TOTAL	**$10,547,690**

FY2009 Research Project Summaries

Project #:	09-1016
Project Title:	Acquisition of a Confocal Laser Scanning Microscope to Enhance the Research Capabilities of University of Missouri at St. Louis
Award Amount:	$281,745
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Xuemin (Sam) Wang, University of Missouri-St. Louis
Collaborators:	Dr. Elizabeth A. Kellogg, University of Missouri-St. Louis; Dr. Colin MacDiarmid, University of Missouri-St. Louis; Dr .Lisa Schechter, University of Missouri-St. Louis; Dr. Teresa Thiel, University of Missouri-St. Louis; Dr. Amy Zanne, University of Missouri-St. Louis; Dr. Bethany K. Zolman, University of Missouri-St. Louis; Dr. Sonya Bahar, University of Missouri-St. Louis; Dr. James K. Bashkin, University of Missouri-St. Louis; Dr. Cindy Dupureur, University of Missouri-St. Louis; and Dr. Jingyue (Jimmy) Liu, University of Missouri-St. Louis

Summary:

This grant sought funds for a confocal laser scanning microscope (LSM) to improve the research capabilities of University of Missouri-St. Louis (UMSL) in areas important to plant science, bioenergy, medical devices, and biomaterials. LSM allows the user to peer deep into cells and tissues and to see internal cellular structures in astoundingly sharp detail. At the same time, preparation of materials to be observed requires minimal time; for many purposes, large pieces of plant or animal tissue can be placed under the microscope and internal structures can be observed directly. LSM has become the industry standard for microscopy; because it is both highly sensitive and time-efficient, it is a critical tool for biological and biomaterial research. With the development of new research programs and hiring of promising new faculty in the science departments at UMSL, the need for a state-of-the-art LSM is becoming necessary and urgent. However, there is no LSM system available on the UMSL campus. The acquisition of a Carl Zeiss LSM 710 will greatly enhance and expand the existing and future research efforts in many areas of biological and biomaterial research. The LSM system will be a versatile instrument that accommodates the diverse research needs in the departments of biology, physics, and chemistry and the Center for Nanoscience.

The availability of an LSM 710 system will improve the research capabilities and faculty's competitiveness for extramural research funding and for translational research. One application of the LSM is to enhance the research activities in several labs in searching for more efficient ways to capture and convert solar energy to produce bioenergy. The instrument and research activities will provide excellent opportunities to integrate frontier research with education to train students and scientists in research areas important to plant science, bioenergy, medical devices, and nanotechnology.

Update: A Carl Zeiss LSM 700 confocal microscope imaging system has been purchased and installed in the Center for NanoScience. Accessories required for imaging acquisition and data analysis have also been purchased and installed. The company has conducted two rounds of training on how to use the instrument and will conduct one more. The availability of the confocal imaging system will improve the research capabilities and education.

Project #:	09-1018
Project Title:	Derivation of Induced Pluripotent Cells from the Pig
Award Amount:	$180,000
Center for Excellence:	Statewide
Lead Investigator:	Dr. Toshihiko Ezashi, University of Missouri
Collaborators:	Dr. Randall Prather, University of Missouri

Summary:
The ability to copy valuable animals in such a way that their merits, for e.g. milk production, semen quality, rate of gain, disease resistance, are maintained in the progeny of the original animal continues to be a problematic and inefficient process. One way to make progress in this area is to create pluripotent cell lines from the skin or some other accessible tissue of the animal, a process that could be achieved without killing or harming the donor animal, e.g. the valuable pig or cow. Their goal is to express a suite of special genes in these cells in such a way that they become reprogrammed and completely undifferentiated.

Such cells are known as induced pluripotent cells and can contribute to all the tissues of the body. However, they can be derived from a relatively small biopsy of ordinary tissues of the adult animal, without resorting to manipulation or loss of a live embryo. They seem to have achieved this end with porcine fibroblasts by expressing a combination of just four genes in addition to supplementing with a drug and a specialized growth factor. If pluripotency can be established in these cells, they will have great value for exact copying the pig by Dr. Randall Prather at the National Swine Center at the University of Missouri. The induced pluripotent cells are likely to provide greater efficiency than established procedures, and should likely mitigate the abnormalities and deaths that are common to animals cloned from differentiated cells, which appear not to be programmed correctly.

There are three aims to the proposal described here, plus a future long term aim for which funding is not presently being sought. Aim 1 is to create several induced pluripotent cell lines and to establish optimal culture conditions and freeze storage conditions for these cells. The second is to demonstrate that the cells are pluripotent according to standard criteria. Such cells, for example, should continue to grow indefinitely without senescing and have a stable chromosome complement throughout this time. They are expected to express genes typical of undifferentiated rather than differentiated cells, yet be capable, given an appropriate stimulus, to differentiate into a multitude of different tissue types, e.g. to nerve cells or liver cell. Aim 3, will compare different combinations of genes for re-programming differentiated pig somatic cells and determine which one is optimal. A final, longer term goal is to collaborate with Dr. Prather to show that these cells can be used to create pigs that are exact copies of the animal that contributed the cells in the first place. The results obtained in the grant are likely to lead to commercial development and allow the investigators to gain large scale federal and industrial funding and additional research stemming from the project.

Update: Successful establishment of pluripotent embryonic stem cells from ungulates, especially pigs, is an important but challenging endeavor. The pig is an attractive species for creating pluripotent cell lines because, unlike the currently preferred mouse model, in its size, anatomy, immunology, and physiology, the pig resembles the human quite closely. We have derived induced pluripotent stem cells (iPSC) from pig fibroblasts by means of retroviral transduction with four reprogramming genes (*OCT4, SOX2, KLF4,* and *c-MYC)*. The results have been published in a prestigious journal (Ezashi, Telugu et al. 2009) and received much attention from the media. This paper demonstrated that the colonies resulting from re-programming had the typical morphologies of ES and iPS colonies from human, expressed "stemness" genes and

transcriptome profile typical of pluripotent cells, as well as a range of proteins, including alkaline phosphatase and cell surface markers characteristic of ESC. They also met the usual criteria of pluripotency, including the ability to form teratomas and embryoid bodies containing tissues representative of mesoderm, endoderm, and ectoderm. The porcine iPSC (piPSC) are capable of dividing more or less indefinitely in culture without senescence and shows high telomerase activity. In other words, the main aim of the grant has been achieved. We are now planning to write proposal to federal and other agency sources to gain funding when the present grant expires.

More recently we have shown that the cells can be stimulated with appropriate growth factors to differentiate into cells resembling neurons, and we are currently attempting to generate cardiomyocytes (heart cells). One problem with the cells in common with other iPSC is that they continued to express the original four genes employed for re-programming, including the two oncogenes *KLF4* and *c-MYC*. Such expression might interfere with directed differentiation and, more importantly increase the possibility of tumor formation if the cells were used as a source of tissue grafts or used to clone pigs. Accordingly, we are now testing methods that employ nonintegrating vectors, particularly the ones described recently (Yu, Hu et al. 2009).

Experiments are underway with Dr. Randall Prather to determine if the cells a) can be used in somatic cell nuclear transfer to clone pigs and b) contribute to adult organs and tissues if incorporated into early embryos. We are also experiment with new methods to create porcine iPSC in which the reprogramming vectors are not retroviruses. This should reduce the chance of the re-programming genes genes being expressed in the cell lines produced. We are also reprogramming cells derived from the umbilical cords of newborn piglets and finding that the process is more efficient.

Ezashi, T., B. P. Telugu, et al. (2009). "Derivation of induced pluripotent stem cells from pig somatic cells." Proc Natl Acad Sci U S A **106**(27): 10993-8.
Yu, J., K. Hu, et al. (2009). "Human induced pluripotent stem cells free of vector and transgene sequences." Science **324**(5928): 797-801.

Project #:	09-1019
Project Title:	Workforce Development and Business Incubation: Animal Health and Nutrition Infrastructure in Missouri
Award Amount:	$285,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Gary Clapp**,** Institute for Industrial and Applied Life Sciences
Collaborators:	Dr. Benjamin Caldwell, Missouri Western State University and Dr. Bern Eichenmueller, Boehringer Inghelheim Vetmedica

Summary:

This proposal targets the Bond Science and Technology Incubator clients, as well as education and training of scientists and engineers who will be preparing for careers in the Animal Health and Nutrition industries in Northern and Western Missouri. Current academic training does not satisfy the specific industrial need. The projected growth in the life science industry will only exacerbate the problem. This proposal seeks to enhance training and educational activities being conducted at the Kit Bond Science and Technology Incubator through additional build out of a laboratory set to function under the current Good Manufacturing Practices (cGMPs) and Good Laboratory Practices (GLPs) regulations. This proposal seeks funding to build and operate a small functional clean room and to purchase additional scientific equipment and casework in support of this resource. The result will create a clean room in an environment that will not only simulate the environment where graduates will work, but could be used as a setting where incubator clients perform early hand filling and/or

finish operations. The rationale for presenting this proposal lies in the cost of operations of a clean room. The cost to operate and/or access a clean room makes this resource difficult to afford and prohibitive for small and start-up firms.

The Institute for Industrial and Applied Life Sciences (IIALS) is presenting this application to the Missouri Life Science Trust Fund Board in an effort to gain support for workforce and economic development in Northern and Western Missouri. The IIALS is a public-private partnership operating as a 501(c) 3 not for profit organization. The stakeholders in the IIALS include Missouri Western State University, Boehringer Ingleheim Vetmedica Inc, IVX Animal Health, AgriLabs, Clipper Distributing, Nestle Purina PTC, Heartland Health, the City of St. Joseph, Buchanan County, the St. Joseph Area Chamber of Commerce, The Wes Remington Family, The Bradley Family Trust and the Messick Trust. The Institute's mission is to promote applied life science activities in the Northern and Western regions of Missouri, inclusive of Kansas City. The Institute further defines its mission, vision and goals into three primary areas of emphasis: Workforce Development, Economic Development, and Advocacy for Animal Health and Nutrition.

The Institute also operates the Kit Bond Science and Technology Incubator and the Missouri Innovation Center of St. Joseph. The IIALS has the advantageous position of being able to offer access to these services and equipment needed by entrepreneurs as they begin to grow and develop their commercial products and ideas for the Animal Health Corridor.

Update: A Modular Clean Room was purchased through VWR International. Delivery and installation of the 3-room clean room; which includes a Class 10,000 Gowning Room, a Class 1000 room, and a Class 100 room, has been completed. While the clean room is not ready or validated for client use, it is being used for training such as the type done in the recent Intro to BioManufacturing class.

Construction of an outer containment and work room for the clean room is nearing completion. All materials and supplies have been ordered and are expected this month. However, we have experienced some delays owing to the nature of equipment and budgeted items availability. Many of the expected support and process equipment have also been purchased. Support processing equipment such as a Freeze Dryer and Pump, Terminal Filling Equipment, Scales, Titrator, Tablet Press and Thermal Analysis Equipment have been purchased or are on order. All remaining monies will be used to validate the operation of the clean room once construction is complete.

Project #:	09-1053
Project Title:	New Medical Materials, Devices, and Instrumentation at the Jordan Valley Innovation Center
Award Amount:	$825,000
Center for Excellence:	Springfield
Lead Investigator:	Dr. Ryan Geidd, Missouri State University
Collaborators:	Dr. Paul Durham and Dr. Matthew Curry, Missouri State University

Summary:

The mission of the Jordan Valley Innovation Center (JVIC) is to improve the translation of research from the laboratory to the end user by providing an environment for entrepreneurship to flourish among scientists through intellectual property incentives. In the Life Sciences, corporate affiliates (including St. Johns Health Systems) help provide the background business information needed to drive high-risk high-reward research toward application. JVIC life science research focuses on medical materials, devices and instrumentation in both the pure and applied research venues.

Located in Springfield on the downtown campus of Missouri State University, JVIC has created a unique environment where corporate research scientists can work side by side with JVIC faculty, staff and students on externally funded programs. JVIC uses its seven senior corporate affiliates, three from within Missouri and four headquartered outside Missouri, to form a caucus that serves as a research and operational advisory board. This Senior Corporate Affiliate Caucus (SCrA) helps guide the research mission of JVIC and its intellectual property (IP), expansion, infrastructure and shared equipment policies. JVIC and its sub-centers operate as a non-profit institute where high-risk/reward research can be pursued without fear of losing commercial IP or patent rights. In addition, JVIC has a close relationship to Springfield Innovation, Inc., a Missouri Innovation Center, which provides further unique opportunities for the commercialization of research programs.

JVIC includes a 75,000 sqft facility called the Roy Blunt JVIC building that includes 4,000 sqft of Class 10, 100, and 1000 clean rooms that specialize in materials synthesis and nano/micro device fabrication. JVIC also houses medical instrument prototyping, medical materials, and microscopy laboratories. In addition to equipping these laboratories, JVIC has purchased over $20M in state of the art materials synthesis and analysis equipment that is utilized by both the corporate affiliate scientists and the JVIC research staff. External research project income has totaled to over $35M since FY03, not including facility renovation grants.

Medical research at JVIC is roughly divided among the physical length scales inherent in the project. At the smallest length scales JVIC is developing well known FDA approved materials systems into morphologies and microstructures that can improve long and short term in-vivo performance. At somewhat longer length scales, in-vitro experiments are performed to develop materials integrated with devices to make passive components active or “smart”. At larger scales, fully integrated instrumentation compatible with existing signals and systems is being built to drive or provide adaptation to existing instruments or facilities. Finally, at the largest length scales, JVIC has specialized super computer and artificial intelligence facilities including a unique 0.46 TFOP (trillion floating point operations per sec) CRAY computer capable of developing models for ultra high speed analysis of vast arrays of medical related information.

Update: JVIC, through our LSRB grant, has established an outstanding model for medical and life sciences technological commercial product acceleration. The grant attracted both corporate sponsors and additional federal dollars to allow companies to pull new products from the research phase into the commercial market at increasing speeds. These private, state and federal investments have leveraged a dramatic downtown redevelopment and an increasing number of high tech jobs. More advanced educational opportunities will be available in the future as advanced manufacturing replaces prototype production.

Project #:	09-1055
Project Title:	Pseudospark Pulsed Plasma X-ray Generation for Portable Medical Devices
Award Amount:	$164,268
Center for Excellence:	Statewide
Lead Investigator:	Dr. Josh Rovey, Missouri Science and Technology
Collaborators:	Dr. Scott Kovaleski, University of Missouri

Summary:

Current medical x-ray devices are large and rely on a high-voltage, high-current electron beam impinging onto a target to emit x-rays (bremstrahlung "braking" radiation). Further, the target must rotate at high speeds to dissipate the power of the beam. In the future, a small, portable xray device is envisioned that can be used on-site, at an accident, on the battlefield, in a clinic, or in the operating room. One potential technology that may achieve this goal is pulsed plasma based electron sources. In these devices, the high-energy electron beam is not space-charge limited, so higher currents at lower voltage and lower power can be obtained.

A pseudospark relies on pulsed current from a stored capacitor to generate a plasma discharge that emits a high-energy, high-current electron beam. Direction of this electron beam onto a target is known to produce EUV and soft x-ray emissions. With the proper scaling of the device geometry and pulse forming network, the pseudospark may also produce hard x-ray emissions that are suitable for medical applications. The benefit of this device is that it has a space-charge neutralized beam that decreases the required power for a given beam voltage (P~V2, as opposed to P~V5/2 for traditional space-charge limited x-ray sources). Since less power is required, the power supply electronics and thermal constraints are relaxed and a smaller device becomes possible.

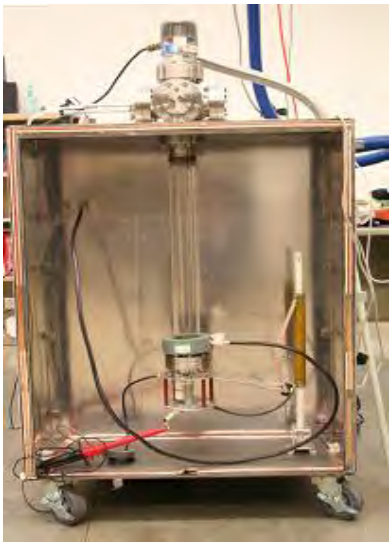
The project consists of a 2 year study to investigate pseudospark plasma discharges for x-ray generation in a portable medical device. The overall objective is to demonstrate pseudospark xray production with the energy and quantity necessary for medical applications. They will combine modeling simulations and experimental testing to complete this objective in three main phases.

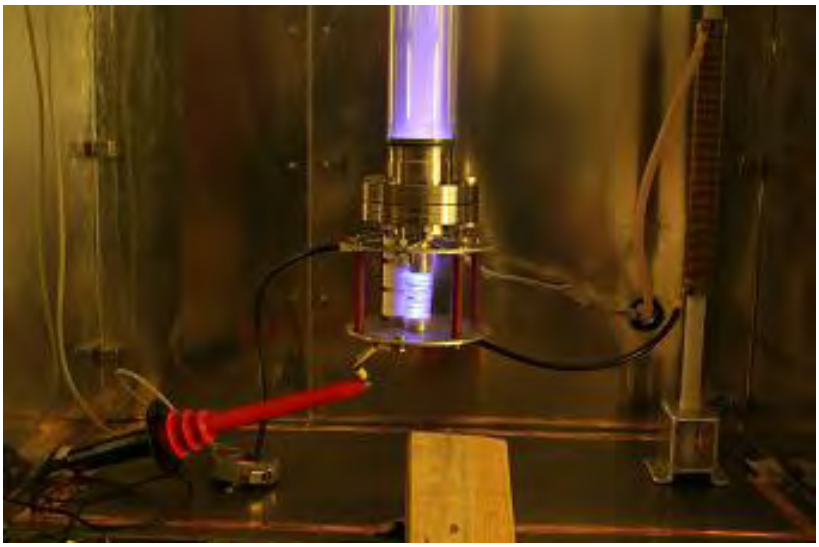
The expected outcome is a set of design criteria (geometry and pulse forming network) for developing a small, portable medical x-ray device that uses a plasma-based pseudospark for xray generation. First, an electron beam investigation will be completed. The initial pseudospark device will be designed based on previous results from a 100 kV device. However, this design will be modified and adjusted based on electron beam propagation and pseudospark operation modeling. Results from this phase will quantify the pseudospark-produced electron beam parameters, such as current, current density, and charge transfer per pulse. The optimum devices from this phase will be selected for x-ray measurements in phase 2.

Phase 2 will focus on x-ray measurements by placing a tungsten target downstream of the pseudospark device. Si PIN and CdTe diode detectors will be positioned to measure the x-ray spectrum. These results can be used to determine the exposure, absorbed dose, and other relevant metrics that will be compared with traditional x-ray tubes. In phase 3, the x-ray measurements will be used to determine which pseudospark devices are viable for medical applications. Specifically, they will determine the pseudospark design characteristics required for medical x-ray production. These characteristics will then be used to suggest a future pseudospark design that should be used in future testing and device development.

Update: This two-year project aims to develop a pseudospark plasma source for biomedical x-ray generation. The benefits of using pseudospark plasma instead of conventional thermionic cathode technology is lower power and smaller size. The pseudospark is a space-charge-neutralized electron source, unlike space-charge-limited thermionic cathodes.

During the first year, we have designed, fabricated, and operated a pseudospark plasma source. Photographs of the experimental setup and pseudospark plasma discharge are shown below. Currently we are operating the pseudospark plasma at voltages up to 40kV, which produce approximately 24 A of electron beam current during a single pulse. Immediate plans for the experimental portion of the project are to increase voltage up to 70kV, relevant for biomedical applications, and measure plasma discharge and beam properties.





In addition to the experimental effort, our colleagues at Univ. of Missouri-Columbia have initiated modeling of the pseudospark plasma. They have completed simulations of a single-gap pseudospark device operating at 2kV using the commercially-available XOOPIC code. The modeling work, along with experimental results were recently presented at the APS Division of Plasma Physics meeting in Atlanta, GA.[1]

[1] Hu, J., Rovey, J. L., Kovaleski, S., "The Research on a High-Energy Pseudospark-Produced Electron Beam," *51st APS Division of Plasma Physics Meeting*, Atlanta, GA, Nov. 2-6, 2009.

Project #:	09-1065
Project Title:	Informatics Research Core Facility
Award Amount:	$1,302,217
Center for Excellence:	Statewide
Lead Investigator:	Dr. Mark McIntosh, University of Missouri
Collaborators:	Dr. Chi-Ren Shyu, University of Missouri

Summary:

The dynamic changes in technology-based research and information generation require sophisticated informatics tools and expertise to analyze massive data sets, and advanced statistical analysis methods to address issues of multi-dimensional experimental design and data analysis. Modern molecular technologies allow system-based investigation of the entire genetic and protein complements of important agricultural species of plants and animals to an extent never before possible. Scientists trained in these

molecular technologies are generating extensive data sets from the experimental examination of the genomes and application of different 'genomics' tools to a variety of plant and animal species that hold significant promise for the next generation of agricultural advances. The obstacle in this quest is the mining of these data sets for that critical piece of information that holds the key to such advances. Most life scientists are not sufficiently trained in the informatics technologies required to manage and interpret these data libraries. This project focuses on establishing a resource team with the technological knowledge and capability to develop and apply informatics tools to animal and plant sciences projects while systematically analyzing and archiving research data sets useful to researchers throughout Missouri and the nation.

Through synergistic collaborations with the MU Informatics Institute, the UM Bioinformatics Consortium and the Office of Research Core Facilities, they plan to create an Informatics Resource Core Facility (IRCF) which will enhance Life Sciences research projects across Missouri. The IRCF will complement other molecular core research activities and new sophisticated, high-throughput instrumentation in genomics, proteomics, metabolomics, imaging and structural biology. The shared resource will allow research teams to access and gain bioinformatics expertise for the design, data management, and analysis of their research projects and a data warehouse to store their research data sets and resulting analyses. The IRCF will also provide an opportunity for bioinformatics faculty to create the next generation of algorithms and tools to address multifaceted questions created by a systems biology approach to today's interdisciplinary life sciences research. The Informatics Resource Core Facility will build our state's life science research capacity and enable researchers to more efficiently and effectively conduct their research and analyze their research results, thus gaining an advantage in garnering additional external funding for their research, and better serving the economic and welfare of Missouri's citizens. With one-time startup funding spent carefully over three years, they will collaborate with researchers to build the on-going costs into their external research grant requests. With this one-time investment, their synergistic team will build a self-sustaining informatics research core facility.

This project directly addresses Missouri's research priorities for the application of basic genomic and proteomic information from laboratory studies toward the enhancement of food animal and agricultural crop production around the state. Anticipated informatics personnel and equipment enhancements will aid to mine plant and animal data sets and identify key molecular determinants of agricultural fitness that have the potential to drive the agricultural industry of the state for the foreseeable future.

Update: The following specific activities have been directed toward meeting the objectives of the award:

1. Computational hardware was purchased to enhance data storage and analysis capabilities related to molecular core technologies. These resources include an additional computational node for the UMBC Q12000X computational platform ($110,460), Isilon IQ system software for data analysis ($69,854) and software support ($49,040). These specific items have been integrated into the UMBC computational framework and made available for IRCF functions.
2. The IRCF Oversight Committee developed job descriptions for the three critical positions staffing the IRCF, Associate Director, Biostatistician, and Data Base Administrator/Data Modeler. The Associate Director position received approval through University administration, was advertised, and candidates vetted by the PI and the Oversight Committee. Two finalists were chosen for campus interviews, conducted the first two weeks of November. The finalist, Dr. Scott Givan from Oregon State University, was chosen based on his 7 years experience with a similar informatics facility at that institution. Negotiations are in the final stages and the candidate is expected to start by March 1, 2010. The Associate Director will then work with the Oversight Committee to fill the remaining two positions.
3. The other two position descriptions are currently being reviewed prior to posting. It is anticipated that all three positions will be filled by Spring 2010 and the IRCF will initiate informatics services.

4. The IRCF Oversight Committee has begun deliberations to (i) establish a fee structure for the IRCF informatics services, and (ii) coordinate with the MU Informatics Institute to establish educational objectives for MUII students that integrate the research services of the IRCF.

Project #:	09-1076
Project Title:	St. Louis Institute for Nanomedicine
Award Amount:	$1,500,000
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Sam Wickline. Washington University
Collaborators:	Dr. Karen L. Wooley and Dr. Dong Qin, Washington University

Summary:

The recent emergence of nanoscience as a key approach to innovation in advanced materials has sparked a similar interest in the application of its principles to the fields of biomedical diagnostics, therapeutics, and basic cell physiology. The overarching goal of the proposed St. Louis Institute for Nanomedicine is to advance the safe and effective use of nanotechnologies to reduce death and suffering from human disease. The global theme that will integrate and focus the planned Institute is *Translation* through development and preclinical evaluation of new nanotechnologies for health care, assessment of safety in production and utilization, facilitation of technology transfer and clinical trials, and education of a new workforce and the public at large. Resources and infrastructure that will be developed to facilitate interdisciplinary implementation of nanomedical devices through collaboration by sponsoring:

1. Research: to expand basic research in nanoscale materials, structures, devices, and pharmaceuticals that may be of benefit for understanding, diagnosing, treating, or preventing human disease;

2. Translation: to guide translation of laboratory breakthroughs into clinical trials by demonstrating safety and efficacy of nanomedical devices and products;

3. Technology Transfer and Commercialization: to foster an entrepreneurial pathway for local commercialization of nanomedical products; and

4. Education and Workforce Development: to train the next generation of scientists and clinicians who will invent and use novel nanomedical products, expand the local talent pool for translation and commercialization, and bring relevant information to the public regarding new developments in nanomedicine.

Specifically, they will create a regional Institute with an inclusive, open network organizational structure: a synergetic environment that should cultivate and enable innovative research directions and translate breakthrough scientific discoveries into practical applications of nanotechnology in the areas of clinical imaging, diagnostics and drug discovery and delivery. They will leverage on their current multi-institutional and multidisciplinary strengths primarily in the development of nanoscale devices to probe and treat cardiovascular disease and cancer and build translational research to generate new commercial opportunities to maximize the regional economic benefits of investments by the State, city, and regional academic institutions and industry partners in medical nanotechnologies.

Update: The St. Louis Institute of Nanomedicine Advisory Board met three times during the spring of 2009, with the Institute's opening ceremony held on July 16, 2009. The event was attended by over 100

people including government, industry and partnering institutions. A Nanomedicine Symposium is being planned for February 2010.

A key long-term commitment of the Institute is the identification and funding of pilot projects to expand the portfolio of nanomedicine ideas and attract new talent to grow the regional nanomedicine infrastructure. An annual request for proposals will be offered to stimulate regional team science by requiring the involvement of at least two co-principal investigators from different disciplines, campuses, or institutions. Awards will be granted for 1-2 years depending on the scope of the project, and will be intended to develop the necessary preliminary data to submit subsequent proposals for continuing support to external funding agencies. Requirements will include a statement of milestones and deliverables, a compelling plan for future grant submissions, and a proposed pathway for clinical translation. The collaborative resources of the Institute will be available to carry out the research for these funded projects. From the grant funds, eight pilot projects were recently awarded funding totaling $280,000. The website for the Institute is currently under construction and can be found at nanomed.wustl.edu/index.html.

Project #:	09-1078
Project Title:	Computational simulation of canine biomechanically induced unicompartmental osteoarthritis: a concurrent multiscale approach
Award Amount:	$556,957
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Trent Guess, University of Missouri-Kansas City
Collaborators:	Dr. James Cook, University of Missouri; Dr. Reza Derakhshani, University of Missouri-Kansas City; and Dr. Ganesh Thiagarajan, University of Missouri-Kansas City

Summary:

In 2005, 32% of Missourians (1.38 million people) reported having doctor-diagnosed arthritis (information collected from the Behavior Risk Factor Surveillance System). In addition, 60% of Missourians over age 65 have been told by a doctor or health care professional that they have some form of arthritis, of which osteoarthritis is the most common. Worldwide, it is suggested that approximately 400 million people suffer from osteoarthritis (data from the National Institutes of Health and The Arthritis Foundation).

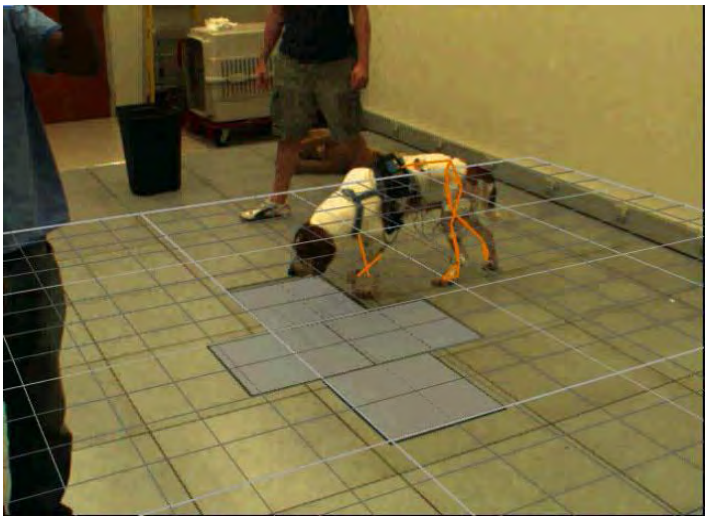
There is no known cure for arthritis and estimates of the total annual cost in the US exceed $90 billion. Osteoarthritis is a debilitating disease that is not completely understood, but evidence links the severity, progression, and treatment of the disease to the mechanical environment in the knee during everyday activities such as walking, running, and stair climbing. The natural response of articular cartilage to insult or injury is an outcome of complex interconnected factors that include anatomy, biology, and muscle forces. The goal of this project is to develop a predictive, computationally efficient, patient level simulation tool of mechanical osteoarthritis indicators. Specifically, a computational model of the canine knee (stifle) that includes surrogate models of cartilage tissue behavior will be combined with musculoskeletal models of movement and validated through in-vivo canine models of osteoarthritis. The computationally efficient cartilage surrogates will predict key tissue indicators of osteoarthritis (shear stress, peak stress, and stress transients) in response to organ level loading and learn from a finite element model solution database.

This work combines the internationally recognized expertise in canine osteoarthritis and tissue engineering of the University of Missouri - Columbia with the musculoskeletal biomechanics expertise and innovative multiscale modeling techniques of the University of Missouri – Kansas City. The project will enhance the reputation and research capabilities of both institutions while developing validated simulation tools of the canine neuromusculoskeletal system that include concurrent tissue level response. This research will

enable the development of patient-specific models that predict tissue level mechanical osteoarthritis indicators during movement and simulation tools where tissue level parameters are incorporated in optimization schemes of muscle activation. This project addresses a key area in osteoarthritis research that has largely been neglected, the role of muscles in osteoarthritis pathomechanics including muscle activation patterns and muscle strength.

Update: The goal of this work is to develop computational models of the canine knee that include surrogate models of cartilage tissue behavior. This model will then be combined with neuromusculoskeletal models of movement and validated through in-vivo canine models of osteoarthritis. This work is a collaboration between the Musculoskeletal Biomechanics Research Laboratory at the University of Missouri–Kansas City and the Comparative Orthopaedics Laboratory at the University of Missouri–Columbia. The project addresses a key area in osteoarthritis research that has largely been neglected, the role of muscles in osteoarthritis pathomechanics. Several graduate students and researchers from UMKC and UMC are working on the project and excellent progress has been made in the last 10 months. Project work progress includes:

1) Mechanical testing to determine material properties of the canine menisci and articular cartilage;
2) Magnetic Resonance Imaging and generation of hind limb bone, cartilage, ligament, and menisci geometries;
3) Preliminary development of knee and musculoskeletal models of the hind limb;
3) Meniscal release procedure to induce unicompartmental osteoarthritis;
4) Gait testing at the UMKC Human Motion lab both pre-surgery and post-surgery;
5) Hind limb testing to validate developed musculoskeletal models; and
6) Development of tissue level finite element models of cartilage indentation testing for tissue level surrogates.



Project #:	09-1101
Project Title:	UMKC Center of Excellence in Mineralized Tissues
Award Amount:	$1,050,196
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Lynda Bonewald, University of Missouri-Kansas City

Summary:

Diseases of mineralized tissues such as bone and teeth or of the muscles that control bone movement result in significant health costs in terms of suffering, loss of work and productivity, and even death. There is a tremendous need for new approaches to treating musculoskeletal diseases. Of the 57.9 million Americans injured annually, more than one-half incur injuries to the musculoskeletal system. The most common bone disease is osteoporosis, which leads to fragile bones that break easily. Hip fractures account for 300,000 hospitalizations per year; 20% of those patients die within a year and 20% are relegated to long-term care facilities such as a nursing home. Associated muscle weakness and wasting compound the consequences of immobility. Biomaterials will be required for the estimated 500,000 joint replacements performed annually, and new surgical techniques and rehabilitation strategies will help speed patient recovery.

Scientists are making important discoveries in the lab that could be used to treat patients with diseases of mineralized tissue. Conditions such as obesity and diseases such as cancer can have a devastating impact on the health of the bones, teeth, and muscles and affect patients of all backgrounds and ages. The United States is facing a national epidemic with regards to obesity. Obesity and inactivity in the population are leading to fragile, weak bones in children and account for more than 300,000 deaths per year. While new discoveries are being made as to how exercise promotes musculoskeletal strength, the population is increasingly sedentary. Injuries, chronic disease, and obesity all result in long-term declines in skeletal and muscle strength. Basic understandings of how bone cells respond to mechanical load and the cross-talk with associated muscles will help to define therapeutic strategies for combating changes in skeletal microarchitecture brought on by inactivity.

Other diseases of mineralized tissue include dental diseases and craniofacial conditions that require procedures ranging from tooth restorations to major reconstruction of facial hard and soft tissues. Children lose more than 50 million school hours and adults close to 200 million work hours each year due to dental visits because of deteriorating oral conditions and dental disease. Improved biomaterials technology is needed to develop new approaches for treatment.

The formal establishment and support of a UMKC Center of Excellence in the Study of Mineralized Tissues would accelerate new discoveries and convert these discoveries into therapies and treatment serving the health and welfare of the residents of the state of Missouri. This Center of Excellence will be structured to leverage significant existing resources to generate a mechanism to increase productivity and funding to a much greater extent over that which would be expected from the individual components and constituents. Funding of the present application would provide seed money for projects with high potential for technology transfer and for interdependent, collaborative projects within the Center of Excellence that will lead to federal grants and support from industry. In summary, this Center will build upon the success of its members to create an environment supportive of creative discovery that will attract prominent researchers and facilitate transfer of discoveries to industry and to the patient.

Update: We have successfully launched the Center website http://cemt.umkc.edu. The Center monthly seminar meetings are the third Wednesday of each month: The UMKC Center of Excellence in Mineralized Tissues Series. The Center Leadership team has established a meeting every third month to discuss the focus and direction of the Center. Two of the Center projects (8 and 11) have received outside funds based on data generated with Missouri Life Sciences Resource Board support.

The goal and purpose of the UMKC Center of Excellence in the Study of Dental and Musculoskeletal Tissues is to accelerate new discoveries and convert these discoveries into therapies and treatment serving the health and welfare of the residents of the state of Missouri. This Center of Excellence is

structured to leverage significant existing resources to generate a mechanism to increase productivity and funding to a much greater extent over that which would be expected from the individual components and constituents. Funding is sought to provide seed money for projects with high potential for technology transfer and for interdependent, collaborative projects that will lead to federal grants and support from industry. The Center strives to create an environment supportive of creative discovery that will attract prominent researchers and facilitate transfer of discoveries to industry and to the patient.

Members of this center, approximately 30, are from the Schools of Dentistry, Medicine, Nursing, Chemistry, and Computing and Engineering. Their total funding is approximately $30 million. The Center has yielded significant returns even though it has only been funded through the Missouri Life Sciences Research Board beginning January 2009. Fifteen pilot projects have received funding after being approved by the internal review committees.

One of these projects entitled "Crosstalk between muscle and bone" generated data that was used in a 'Grand Opportunities' application to NIH. This proposal received a score of outstanding (25) and a review stating that "The proposed project will have a high potential impact on studying the relationship of muscle and bone and open new avenues of research into the population with both muscle and bone disease" and has been funded for $1,077,000.00 for two years.

Secondly, another project initiated through the Center grant entitled "The Genetic Influence of the Immune System on Periodontal Disease", PIs: Hong-Wen Deng, PhD SOM and Liang Hong, DDS, PhD, SOD has received funding for a pilot study "Gene expression study of monocytes for periodontitis" totaling $173,156. The objective of this study is to identify the genetic components that are responsible for the detrimental effects of the immune system on periodontal disease. This is already a significant return on the investment by the MLSRB.

Project #:	09-1105
Project Title:	Acquisition of Metabolomics Platform for Metabolic Engineering
Award Amount:	$894,993
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Leslie Hicks, Donald Danforth Plant Science Center

Summary:

The ability to detect, identify, and characterize biomolecules remains an essential component in the elucidation of complex cellular processes. Progress in the field of analytical instrumentation development continually advances current capabilities and the ease by which these efforts can be carried out. The purpose of this proposal is to seek funding for two Agilent LC-mass spectrometers, a 6200 series accurate mass TOF and a 6500 series accurate mass QTOF, to support research of scientists at the Danforth Center as well as their partner institutions and current collaborators. The combination of these two high performance instruments will establish a *robust, high-throughput metabolomics platform* with high resolution, high dynamic range, and sub-picogram sensitivity. High mass accuracy MS capabilities are essential for full characterization of molecules in metabolic engineering efforts and the addition of this instrumentation at the Danforth Center will provide easy access to a large number of scientists in the St. Louis region which are in need of this advanced technology.

The Donald Danforth Plant Science Center is an independent, non-profit research institution conducting interdisciplinary plant science research in genetics, biochemistry, cell biology, physiology, and structural biology. Established in 1998, the Danforth Center is a 170,000 sq. ft facility constructed on a 40-acre site

located in St. Louis, MO. Dr. Roger Beachy is the founding president; there are currently 18 principal investigators; and the Center is actively recruiting three new senior PIs. The Danforth Center includes state-of-the-art research laboratories, green houses, growth chambers, and three core scientific facilities that service the needs of scientists in the areas of microscopy, tissue culture, and proteomics/mass spectrometry. It has strong educational and research partnerships and has established active international research and training programs. The Danforth Center provides infrastructure that enables training of scientists, postdoctoral fellows, and graduate/undergraduate students, accomplishing one of the guiding tenets of the Danforth Center charter: to provide a world-class institute where scientists from around the world can learn, train, and conduct research to answer some of the most important questions in plant biology.

The research projects described in this project require a platform that suits the various needs of multiple plant biologists. For some projects, the platform must enable determination of elemental composition for structural elucidation of small molecule metabolites, where as identification and characterization of metabolites changing due to a defined system perturbation will be necessary for other projects. The overall superior sensitivity, dynamic range, and mass accuracy of instruments available for these studies will be essential for successful completion of all the projects and thus in securing other research funding. The success of this proposal will positively impact research in the areas of natural product biosynthesis, plant/microbe interactions, plant lipidomics/sphingolipidomics, and metabolic engineering for enhanced biofuel systems. The requested instrument will allow plant biology researchers at the Danforth Center and collaborators to further expand their research scope into the interface of biology and chemistry and promotes the creation of cross-disciplinary specific aims and collaborations that will facilitate a macroview analysis of specific projects while deriving systems-wide information.

Update: With the award of this grant for the purchase of instrumentation, the PI negotiated with the instrument manufacturer and leveraged the purchase of additional and more advanced instrumentation with the grant funds. The grant originally requested the purchase of one TOF and one QTOF instrument for the purposes of establishing a robust, high throughput metabolomics platform. Through the negotiating process, the TOF instrument was upgraded to a second QTOF instrument (which has the added benefit of MS/MS) and additionally - a GCMS and auxiliary separations equipment was purchased to further enhance our capabilities in the metabolomics area and facilitate our method(s) development. Major instrumentation was ordered in January 2009 and delivered/installed from March-May 2009. Instrument training was carried out in June 2009 and troubleshooting / stabilization of instrumentation was performed from July 2009-September 2009. Currently, method(s) development (including sample preparation, instrument and data analysis methods) is in progress on all instrumentation for the robust, high-throughput non-targeted profiling of metabolites. These platforms will be heavily used as an enabling technology in the recently funded DOE-EFRC Center for Advanced Biofuels Systems (CABS) (Hicks, co PI) for rational engineering in biofuels research.

Project #:	09-1106
Project Title:	Drought Simulators Critical to Translational Research in Plant Science
Award Amount:	$1,558,125
Center for Excellence:	Statewide
Lead Investigator:	Dr. Felix Fritschi, University of Missouri
Collaborators:	Dr. Robert Kallenbach, University of Missouri; Dr. Grover Shannon, Delta Research Center; Dr. Stephen Anderson, University of Missouri; Dr. Deborah Finke, University of Missouri; Dr. Robert Kremer, University of Missouri; Dr. Randall Miles, University of Missouri; Dr. Henry Nguyen, University of Missouri; Dr. Melvin Oliver, University of Missouri; Dr. Craig Roberts, University of Missouri; Dr. David Sleper, University of Missouri; Dr. William Stevens, University of Missouri; Dr. Kelly Tindal, University of Missouri; Dr. William Lebold, University of Missouri; Dr. Allan Wrather, University of Missouri; and Dr. Xi Xiong, University of Missouri

Summary:

Water is a finite resource that is in great demand for a wide variety of reasons, including domestic, industrial, leisure, and agricultural uses. In light of population increases and greater demands for non-agricultural uses, more and more emphasis will have to be placed on efficient use of water resources available for plant production. Presently, facilities to reliably examine plant responses to drought under field conditions do not exist in Missouri. However, to translate research findings developed in controlled environment facilities to field conditions, the ability to manage the timing, duration, and intensity of water deficit stress under field conditions is essential. The drought simulators currently under development are critical in bridging the gap between laboratory based experiments and conditions encountered in the field. Once completed, Missouri researchers will have access to a unique network of drought simulators that will allow them to address a broad range of topics, including 1) identify new germplasm with increased drought tolerance, 2) identify underlying genetic mechanisms that control drought tolerance, 3) uncover and evaluate physiological mechanisms conferring greater drought tolerance, 4) develop and evaluate cultural practices to increase crop productivity and reduce environmental impact, and 5) study the influence of water deficit stress on soil-plant-insect and soil-plant-pathogen interactions.

Update: To date, site selection for the construction of two drought simulators has been finalized based on rigorous selection criteria. The exact location of the two additional modules awaits final decision. Detailed plans for the drought simulator modules have been developed and are being finalized. Construction on the first two modules is scheduled to be initiated early in 2010. The drought simulators under development will benefit major agricultural commodities in Missouri, and will place Missouri scientists at the national forefront of drought-related translational research. This unique facility will lead to research advancements to improve food security, increase productivity of bioenergy crops which will reduce dependence on foreign oil, and increase profitability for farmers while protecting environmental resources, protecting water quality, and increasing water use efficiency

Project #:	09-1117
Project Title:	Advanced Cardiovascular Stent incorporated with Nitric Oxide Delivery System
Award Amount:	$540,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Chi Lee, University of Missouri-Kansas City
Collaborators:	Dr. Hai-Lung, Missouri S&T; Dr. Richard Hopkins, Children's Mercy Hospital; and Dr. Yungyun Lee, University of Missouri-Kansas City

Summary:
Cardiovascular stents are metal scaffolds placed in a narrowed atherosclerotic artery to keep the vessel open by providing structural support and prevent it from re-occluding, a condition called restenosis in which endothelial cell growth proliferates around the device as part of the body's natural wound-healing response and impedes blood flow. Most bare metal stents are reported to cause restenosis after a few months of surgery and thus newly developed stents are covered with inhibitory agents to prevent restenosis. Even though the drug-releasing coatings for stents are an exciting protective device that can provide an enormous clinical benefit, the drug coating may peel and delaminate from the metallic surface of the stent due to poor adhesion between the coating and the stent surface. The coating delamination can potentially lead to embolism, acute thrombosis, inflammation and non-uniform delivery of drugs.

In this proposal, they will attempt to reduce restenosis and improve the biocompatibility of a metal scaffold (MS) with two nanotechnology approaches: i) pattern modification by the nano/micromachining technique with a femtosecond laser (FSL) and ii) the development of microparticles (MP) containing Nitric Oxide (NO) using the double emulsion method. The key to this proposed study is the partnership of interdisciplinary scientists in pharmaceutics, engineering, cardiology, advanced proteomics, pharmacological and computer sciences, which seems to be an ideal approach for biomedical research. This proposal is highly innovative in that they will develop the MS with different patterns of the groove in its outer surface which can firmly retain NO loaded MP and exert a controlled release rate of NO according to the loading conditions.

As heart-related diseases remain the most prevalent cause of death in the world, a continuous and controlled supply of NO through MS built with nanotechnology will greatly relieve the long-term risk of cardiovascular diseases. It was also reported that heart disease was the leading cause of death accounting for 16,708 deaths (about 30%) of the Missouri state's deaths in 2002 (National Vital Statistics Report 2004; 53(5)). Therefore, MS built with nanotechnology shall benefit thousands of patients under cardiovascular complications in the state of Missouri by reducing the burden of heart disease and stroke, and promoting activities that can be implemented in health care, communities and schools.

Update: We, as a multi-disciplinary team, have successfully performed most of proposed aims scheduled in the first year of this project. We are sure that the result of proposed aims scheduled in the first year of this project shall benefit thousands of patients under cardiovascular complications in the state of Missouri by reducing the burden of heart disease and stroke, and promoting activities that can be implemented in health care, communities and schools.

The major hypothesis of this proposal is that a metal scaffold (MS) built with nanotechnologies (i.e., i) loaded with microparticles (MP) containing nitric oxide (NO) prodrugs and ii) competently positioned at the grooves (i.e., produced by nano/micromachining techniques with a femtosecond laser (FSL)) will reduce restenosis and improve biocompatibility. To test this hypothesis, we will establish the following objectives:

Objective I: We will develop the MP incorporated with NO prodrugs, diethylenetriamine diazenumdiolate (DETA NONOate; D-N) (high 5 %, medium 2.5 % and low doses 1% w/w), and evaluate their loading efficiency and compatibility in a MS based on groove patterns (i.e., varying sizes and numbers) produced by a FSL at the surface of the stent. We will use the simulated blood flow system for assessment of compatibility (i.e., fitness and stability) of a MP positioned in the MS with varying groove patterns and the release rates of NO from the MP into the intima layer.

Objective II: To elucidate the mechanism behind the NO mediated prevention of the MS induced restenosis, we will evaluate the effects of the MS loaded with MP as compared with the control on i) intima-to-media ratio (i.e., as an index of restenosis) in the rabbit models (1, 2 and 4 wks), ii) the changes in the cyclic GMP (cGMP) level (i.e., as an index of improving endothelial function) in the porcine aortic valve interstitial cells (PAVICs) assessed at 6 hr, 12 hr and 24 hrs after an application of the MS, and iii) the cell cytotoxicity of PAVICs (i.e., as an index of biocompatibility) assessed at 6 hr, 12 hr and 24 hrs after an application of the MS.

Objective III: We will further establish the relationships between the degree of restenosis (i.e., intima-to-media ratio), the properties in the pattern groove (i.e., size and number), the NO release rate and changes in the cGMP level using a data mining approach to accurately predict the pharmacological efficacy of the MS based on the groove pattern and NO loading conditions in the MS.

Project #:	09-1128
Project Title:	Targeting Plasminogen Activator Inhibitor-1 to Inhibit Restenosis
Award Amount:	$815,625
Center for Excellence:	Statewide
Lead Investigator:	Dr. William Fay, University of Missouri
Collaborators: Dr. Douglas Bowles, University of Missouri; Dr. Dmitri Baklanov, University of Missouri; Dr. Daniel Lawrence, University of Michigan; and Dr. Brian Wamhoff, University of Virginia	

Summary:

Missouri is among the states with the highest incidences of cardiovascular disease, ranking 8th in 2005. Many patients with coronary artery disease (CAD) undergo percutaneous coronary intervention (PCI), a procedure in which a cardiac catheterization is performed and a stent is placed in a coronary artery at the site of a stenosis (blockage) to improve blood flow. Most patients receive stents that are coated with drugs designed to prevent restenosis--i.e. the re-growth of the blockage within several months after the initial procedure. Drug-eluting stents inhibit growth of cells within the wall of the artery that cause restenosis. However, within the past few years it has been discovered that drug-eluting stents, in the process of inhibiting restenosis, also inhibit the normal repair of the inner lining of the artery (i.e. the endothelium), thereby predisposing the artery to thrombosis, or abnormal blood clotting, which can lead to heart attack and death. The overall objective of this proposal is to develop new strategies to prevent restenosis without increasing the risk of thrombosis.

The project will study novel compounds that inhibit the function of plasminogen activator inhibitor-1 (PAI-1), a key blood clotting protein. These studies will involve the use of pigs, which are widely regarded as the best animal model to test and develop potential treatment strategies for heart disease in humans. The proposed program is multi-disciplinary in nature, involving collaborations between physicians and basic scientists of the University of Missouri-Columbia School of Medicine and College of Veterinary Medicine, as well as investigators at the University of Michigan and University of Virginia. They anticipate that the data generated from the proposed studies will lead to submission of a Small Business Innovation Research (SBIR) application to the National Institutes of Health focusing on development of new compounds to inhibit restenosis in humans without promoting thrombosis. The University of Missouri-Columbia is the only university in the state, and one of the few in the nation, with a medical school and veterinary school located on the same campus. The collaboration between medical- and veterinary based investigators is a major strength of the proposal. This work has great potential for developing pig models of human diseases that will lead to new treatment strategies for patients. They anticipate that their studies will have substantial

commercial potential, since new approaches are needed to prevent restenosis without promoting thrombosis. Given the high prevalence of CAD in Missouri, the proposed studies are strategically aligned with the state's health care, economic development, and research opportunity objectives.

Update: We have made significant progress in all 3 specific aims since initiation of funding.

Aim 1 is to study the efficacy of PAI-1-R, a recombinant mutant of plasminogen activator inhibitor-1 (PAI-1), in inhibiting intimal hyperplasia after coronary angioplasty in pigs. To date, we have studied 26 pigs. Our collaborator, Dan Lawrence, PhD, University of Michigan, has been providing us with PAI-1-R. In initial studies, we have used two commercially available coronary balloon catheters to deliver PAI-1-R. We also have infused PAI-1-R directly into the coronary artery via an indwelling catheter, and we have infused PAI-1-R systemically via an indwelling jugular vein catheter. We have demonstrated that PAI-1-R can be infused successfully via catheters to balloon-injured coronary arteries. We have used immunohistochemisty to demonstrate that infused PAI-1-R is retained within the wall of the artery for up to two weeks. We have shown that PAI-1-R infused systemically circulates in high concentration for at least two hours after infusion. We have performed morphometric analyses of injured coronary arteries retrieved 2 weeks after balloon injury and administration of PAI-1-R or vehicle control. In our small pilot studies, each involving a different method for delivering PAI-1-R, we have observed a consistent trend towards reduction in neointima formation in arteries treated with PAI-1-R, as opposed to control arteries treated with infusions of saline. We find these results encouraging, and we are about to embark on larger experiments to test the hypothesis that PAI-1-R inhibits intimal hyperplasia after balloon angioplasty of pig coronary arteries.

Aim 2 is to characterize stents coated with PAI-1 targeting compounds. We have purchased a stent coating apparatus with funds provided from this grant. We have coated three commercially available bare metal stents with PAI-039, a low-molecular-weight PAI-1 inhibitor provided to us by Dr. Lawrence. Our collaborator, Brian Wamhoff, PhD, University of Virginia, has been assisting us with the development of strategies for coating and analyzing stents. We have begun to study the release of PAI-039 from coated stents. We will be using high performance liquid chromatography (HPLC) to study the kinetics of release of PAI-039 from stents. We will be using a PAI-1 immuno-activity assay to determine if PAI-039 released from stents retains biological activity against PAI-1.

Aim 3 is to define mechanisms by which PAI-1 targeting compounds inhibit vascular smooth muscle cell (VSMC) migration in vitro. We have completed a series of experiments in which we have determined that the levels of expression of PAI-1 and vitronectin by VSMC play critical roles in determining the rates of migration of cells through 3-dimensional collagen. We have used recombinant PAI-1 mutants to determine specific mechanisms by which PAI-1 can both accelerate and inhibit VSMC migration through collagen. A manuscript summarizing our findings has been submitted for publication. We have begun to study the effects of PAI-039 on VSMC migration in vitro, with our preliminary results suggesting that PAI-039 is a potent inhibitor of VSMC migration.

An important overall objective of our proposal is to develop a small business whose mission is to test compounds with potential therapeutic efficacy in an animal model with strong predictive value of clinical efficacy in humans. Drs. Fay, Bowles, and Wamhoff have had regular in-person and telephone meetings to discuss this objective. Drs. Fay and Bowles also met recently with Wayne Alexander, PhD, University of Missouri Office of Technology Management and Industry Relations, to brainstorm on initial strategies to develop a business plan.

In summary, we have made substantial progress in our studies. We are excited about our initial data, and we look forward with great enthusiasm to continuing this research graciously funded by the Missouri Life Sciences Research Board.

Project #:	09-1148
Project Title:	Optimization of Camelina as a nonfood production platform of value-added biotechnology products.
Award Amount:	$593,564
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Eliot Herman, Donald Danforth Plant Science Center
Collaborators: Dr. Roger Beachy, Donald Danforth Plant Science Center; Dr. Monica Schmidt, Donald Danforth Plant Science Center; and Dr. Gene Stevens, Delta Research Center	

Summary:

They propose to develop *Camelina sativa* as a nonfood industrial crop for Missouri farmers and industrial users. Ideally, a commercially successful industrial crop is one that produces multiple products, each with its own value stream. For example, a plant that produces biodiesel, industrial enzymes, and polymers for plastics will have greater value than one that only produces a single product. The new crop must be adapted to growth and high yields suitable for Missouri farmers. Finally, the crop should be developed to reduce unwanted spread and growth of proprietary materials. This is a collaborative project that brings together basic sciences, biotechnology, and plant breeding. Furthermore, the outcome of the project is of significant interest to Metabolix Inc., as part of their ongoing research. If successful, this project will also lead to the growth of life sciences companies in the State.

Update: The specific goals of this project are to:

(1) Develop the agronomic potential of *Camelina* as a Missouri crop (Dr. Gene Stevens, Delta Center, University of Missouri). A wide range of *Camelina* germplasm will be evaluated by applying standard agronomic experimental variables (effects of nutrients, day length, soil types, etc on growth and yield potential). The variety(ies) selected with high yield and adaptability to Missouri conditions will be potential targets for genetic modification and commercial development.

Progress goal #1: Field trials were conducted at Novelty, MO, in Columbia, MO and in Portageville, MO. Experiments were conducted to evaluate the effects of nitrogen rate and seeding rate on Camelina yields. The experimental design for each test was a randomized complete block with four replications. Disease infestation was found to be a major problem for camelina growth in Missouri. Disease severity varied between sites. All plots were killed in Columbia, MO due to infestation. These infestations were most likely caused by *Botrytis cinerea* (botrytis blight) or *Sclerotinia sclerotiorum* (Sclerotina stem rot) with symptoms beginning at bloom growth stage. Fungicides will be needed to optimize camelina production in Missouri. In the seeding rate experiments, yields increased linearly as seeding rate increased. Yields were much higher at the Novelty location than at Portageville. In the nitrogen rate experiments, yields at Portageville, MO were highest with a N rate of 67 kg N ha^{-1}. No further yield benefit was achieved by applying 100 kg N ha^{-1}. However, at Novelty, MO yields increased as N rate increased up to 100 kg N ha^{-1}. Transplanted accession plots were visually rated throughout the growing season to identify the most vigorous cultivars growing in each climate. A cultivar from the former Soviet Union was the highest yielding accession despite the heavy disease infestation at Portageville, MO. The initial crop cycle has identified some possible problems, disease, as well as a potential advantageous cultivar. With additional field tests MO optimized cultivars and these will be transferred to the Danforth Center for evaluation as transformation platforms to produce biotechnology traits.

(2) Establish a compositional dataset o*f C. sativa.* Proteomic studies will be conducted to identify the major and minor seed proteins of *Camelina* and high throughput sequencing technologies will be applied to create an EST data set from seeds in early to mid maturation. This information is required to create the transgenes that will reduce the abundant proteins in seeds and lead to a pathway through which to 'replace' endogenous proteins with high-value proteins.

Progress goal #2: The initial proteomic assessment of Camelina seeds has been accomplished. Total soluble seed proteins have been separated by two-dimensional isoelectric/size fractionation electrophoresis and the major protein spots identified by trypsin digestion and mass spectroscopy fractionation and identification of the proteins. The close relationship of Camelina to Arabdiopsis and Brassica has aided in the identification of seed proteins. This has resulted in creating a proteome map of Camelina with the major proteins identified and the relative abundance determined. The proteome map is essentual to determine the changes that occur with the expression of foreign protein genes in Camelina and to quantify the accumulated protein as has been accomplished and reported for goal #3.

(3) Remodel composition of *C. sativa* seed proteins. They will apply a proven approach to engineer the composition of soybean seeds (developed by co-PIs, Drs. E. Herman and M. Schmidt) to suppress major seed proteins of *Camelina* and replace them with one or more target protein(s) of commercial interest. The general approach is based upon gene-specific silencing of target genes and selective expression of genes and subcellular accumulation of target proteins in transgenic seeds. If successful, this will lead to high levels of target proteins (goal: >10% of total seed protein).

Progress goal #3: The expression of a test model protein in Camelina has been accomplished by coPI and PI Schmidt and Herman to determine a base level of protein accumulation that occurs without re-allocation of the protein sink. In this experiment with soybean seeds the base level averaged 2% however with Camelina the base level was determined to be 6% of the protein, already much of the way toward 10% proposed for this project. The next steps in this project are underway with the September 09 hiring of a postdoctoral associate who has produced constructs to suppress the endogenous storage proteins of Camelina and are in the process of being transformed into Camelina plants. Once suppression of the endogenous proteins is achieved then the expression of the foreign proteins will be merged into Camelina lines that are expected to exceed the 10% foreign protein goal of this project.

(4) Develop and apply a system to secure intellectual property and reduce unwanted spread of transgenic seeds. A new system is being developed (Dr. R. Beachy) to create hyperdormant seeds that will not germinate unless induced by a chemical gene switch system. This system will be applied to *C. sativa* varieties selected in (1) and (2).

Progress goal #4: In the past several months the constructs needed to test the potential of chemical regulation of seed germination have been produced. These constructs include the gene regulator and protein used to produce a permissive seed germination trait, the gene regulator and protein under the control of a chemical gene switch and other controls. These constructs are being progressively transformed into Camelina with the first set of constructs regenerated into plants after antibiotic selection for the transgenic gene insertion. The segregation pattern and copy number of inserted genes is being investigated in the first transgenic lines produced and this analysis will be expanded as additional lines are selected for the other gene constructs.

The anticipated outcome/product of this pre-commercial project is to develop an over-winter crop for Missouri farmers that will produce high value non-food proteins, oils and other products.

The initial advances made by all four coPIs in the three partner laboratories has this project on track and all of the coPIs are enthusiastic about its potential coming to fruition to develop a new biotechnology crop for Missouri. During the next year as traits and lines are developed we will begin to stack goals 1,3, and 4 together to complete the development of the new biotech traits in Camelina lines developed for Missouri agriculture. If successful, this project will also lead to the growth of life sciences companies, such as Metabolix Company, in the State.

FY2009 Funding Summary – Commercialization

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
09-1011	Molybdenum-99 / Technetium-99m Processing Facility At MURR	Dr. Ralph Butler	University of Missouri/Reactor	Equipment	$1,097,761
09-1024	Translational Development Center	Marcia Mellitz	Center for Emerging Technology/SLU	Equipment	$520,000
09-1034	Photoacoustic detection of circulating melanoma cells in blood	Dr. John Viator	University of Missouri	Project Proposal	$407,789
09-1177	iPrep: Ophthalmic Povidone-Iodine Antiseptic Formulation	Dr. Wendell Scott	St. Johns Medical Institute	Project	$574,450
				TOTAL	**$2,600,000**

FY2009 Commercialization Project Summaries

Project #:	09-1011
Project Title:	Molybdenum-99 / Technetium-99m Processing Facility At MURR
Award Amount:	$1,097,761
Center for Excellence:	Statewide
Lead Investigator:	Dr. Ralph Butler, University of Missouri
Collaborators:	Dr. John Robertson, University of Missouri

Summary:

The University of Missouri Research Reactor (MURR) Center has been working to develop the methodology and facilities for domestic production of molybdenum-99 (Mo-99) from low enriched uranium (LEU), with the objective of meeting half of the nation's medical need for its daughter product, technetium-99m (Tc-99m). In the United States alone, Tc-99m is used in ~35,000 procedures each day. Collaboration with and funding from the Department of Energy has focused on developing new starting material that will yield Mo-99 that not only meets FDA specifications, but also addresses the global issue of nonproliferation and secures a sizable domestic supply of this critical radiopharmaceutical for medical imaging. In parallel with methodology and process design are the design and construction of a production facility addition at the MURR Center.

The MURR Center is seeking $1.3 million for the architectural and engineering design of a Mo-99/Tc-99m processing facility. Having commercial-scale Mo-99 processing capabilities for the production of Tc-99m at the MURR Center will ensure a significant domestic supply for patient use, clinical trials and new R&D applications of a proven radioisotope. As there is no current U.S domestic production of Mo-99, the State of Missouri would become the sole U.S. producer of this critical radioisotope. In meeting half of the U.S. demand, the project would generate annual gross revenues of $30 to $40 million.

Technetium-99m

Technetium-99m (Tc-99m) is the most commonly used medical radioisotope in the world. Over 30 different radiopharmaceuticals use Tc-99m for imaging and performing functional studies of the brain, myocardium, thyroid, lungs, liver, gallbladder, kidneys, skeleton, blood and tumors. It is estimated that more than 70% of all nuclear medicine procedures use Tc-99m, and ongoing research is being carried out to develop even more applications for this isotope. Tc-99m does not occur naturally; because it has a short half-life of 6.02 hours, it has to be continuously produced and cannot be stockpiled. Fortunately, Tc-99m is the decay product (daughter) of molybdenum-99 (Mo-99) which has a half-life of 66 hours. The isotope is made available to physicians and patients by distributing Mo-99/Tc-99m generators to nuclear medicine facilities on a weekly basis.

Production of Mo-99/Tc-99m

Most of the Mo-99 produced today is from the neutron irradiation of high enriched uranium (HEU) targets at reactors in Canada, Europe, and South Africa. After neutron irradiation, the HEU targets are removed from the reactor and chemically processed to recover the Mo-99. The Mo-99 is shipped to a facility that loads the Mo-99 on to an aluminum oxide column known as a technetium generator. The generator manufacturer ships the loaded generator to a nuclear pharmacy, which recovers the Tc-99m by passing saline solutions through the generators. The nuclear pharmacist formulates the drug products, by adding the appropriate amount of Tc-99m solution to a freeze-dried kit. The Tc-99m pharmaceutical is drawn into a shielded syringe and transported to the hospital for injection into the patient by a nuclear medicine physician. Covidien in St. Louis is one of only two licensed Tc-99m generator manufacturers in the U.S.

Update: The University of Missouri Research Reactor (MURR) Center is working towards becoming a large scale supplier of molybdenum-99 (^{99}Mo) through the fission of low enriched uranium (LEU) targets in the reflector region of the reactor. Feasibility studies indicate that MURR could supply one-half of the US nuclear medicine community's need for ^{99}Mo and its decay daughter product technetium-99m (^{99m}Tc). The intended purpose of the $1,097,761 grant is to assist MURR in the development of the detailed design for the facilities necessary for the handling of irradiated LEU targets and the ^{99}Mo extraction process.

The MLSRB grant has supported the development of an enhanced conceptual design and technical project plan that is being used to solicit both public and private sources for the additional funding necessary for the detailed design, licensing, and construction of the facilities. The University of Missouri-Columbia has provided matching funds of $250,000 in support of this project. Without the MLSRB grant the matching funds from the University's PRIME Fund would not have been possible. Through October, PRIME funds have paid for $50K in S&W and benefits, and expenses reported on the LSRB grant include $66.7K for S&W and benefits and $300K for operating expenses including subcontract payments.

The MLSRB grant has supported the development of financial and technical plans including project schedules, work breakdown structure, and enhanced conceptual design including layout of the facility. In general the MLSRB grant has supported the design development work of the LEU targets including target performance yields and failure modes, the irradiation rigs for the targets in the reactor reflector region, the

chemical process for dissolving the LEU targets and extracting the ^{99}Mo, and initiation of the necessary safety analysis. In addition, the grant has supported the development of a draft licensing plan and presentation material for an initial meeting held on May 21, 2009 with the US Nuclear Regulatory Commission regarding the licensing of the facilities. The grant has supported several technical meetings and numerous conference calls of the conceptual design and licensing team.

Below are the conceptual renderings of the ^{99}Mo facility.

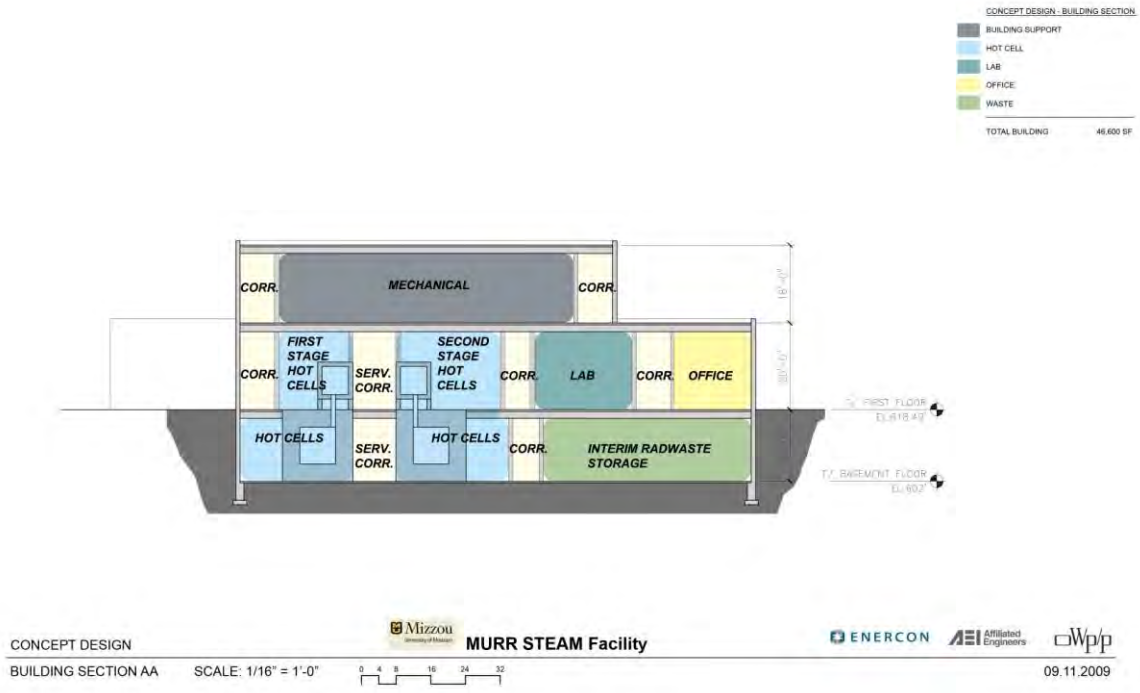


Figure One: Conceptual Design – Section

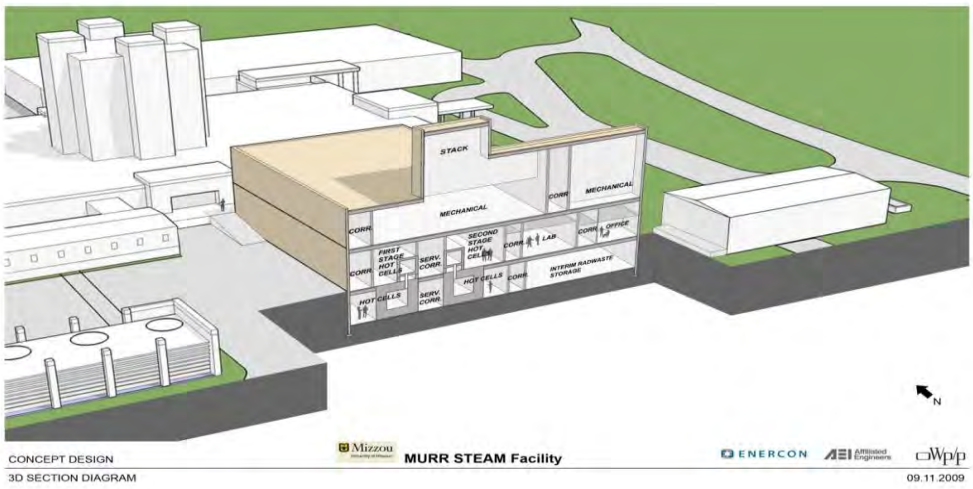


Figure Two: Conceptual Design – 3-D

Project #:	09-1024
Project Title:	Translational Acceleration Center
Award Amount:	$520,000
Center for Excellence:	St. Louis
Lead Investigator:	Marcia Mellitz, Center for Emerging Technologies

Summary:

This grant will create a Translational Acceleration Center for life science start-up companies involved in proof-of concept research. Collaborating on the project are the Center for Emerging Technologies, Washington University, University of Missouri-St. Louis, St. Louis University and BioGenerator. The St. Louis region has the potential to be in the top ten life science regions, but only if commercialization activity is greatly increased.

This will project will fit out and equip 300 sq ft. modules of wet lab research space and associated office space in the CET Building III which is currently being developed. The lab fit out will include benches, sinks, fume hoods, casework, and advanced telecommunications capacity. CET will provide these companies with extensive support services through the company creation and development phases. The leases will be short-term and priced affordably so as to easily enable the transition from university lab to company creation. This project will be designed to serve new start-up companies with very limited funding and the need to use their resources for product development rather than lab fit out.

This project is an important component in building the life science capacity of Missouri by developing physical facilities, support services, and a mechanism of collaboration to transform university discoveries into commercially viable innovative products. Overcoming the "Valley of Death", that critical transition from university bench to company formation and successful early stage growth, is a barrier facing many institutions and organizations across the country. Although scientific breakthroughs are important and essential, it is bringing them to market that creates a new 21st century industry base for the state and its long term economic advantage.

Update: Planning for the Translational Development Center (TDC) has broadened due to new developments for CET. CET is currently engaged in discussions with regional life science leaders concerning expanding CET's role regarding technology commercialization and translational research activities to the wider St. Louis area. With increased scope of mission for CET the need for the Translational Development Center would be intensified and the criteria for selection expanded. This new role for CET will involve a closer relationship with CORTEX which is currently engaged in the selection of a new president. CET expects that CORTEX will be involved in the development of CET's Building III (housing the TDC) and therefore once the new president is on board further planning for the TDC will continue in light of these new developments.

Project #:	09-1034
Project Title:	Photoacoustic detection of circulating melanoma cells in blood
Award Amount:	$407,789
Center for Excellence:	Statewide
Lead Investigator:	Dr. John Viator, University of Missouri
Collaborators:	Dr. Paul Dale, Dr. Scott Holan, and Dr. Luis Polo-Parada, University of Missouri

Summary:
Melanoma is the deadliest form of skin cancer and has the fastest growth rate of all cancer types. In the U.S., the lifetime risk is about 1 in 55, while other parts of the world have even greater risks. Early surgical resection of melanoma is the best avenue of therapy. However, for those cases where the lesion progresses and spreads, monitoring of metastatic disease is crucial for positive clinical outcomes.

Detection of circulating melanoma cells (CMC's) in human blood and lymph systems has the potential to find early metastasis and monitor therapy. They have detected CMC's by generating photoacoustic waves, or laser induced ultrasound, in melanoma cells suspended in saline and in single CMC's in blood samples from advanced melanoma patients. They will improve their current photoacoustic detection device by incorporating an optical reactance acoustic sensor in their apparatus and by implementing a two color laser method for increasing the ability to specifically find melanoma. These improvements will increase accuracy for single melanoma cell detection of lightly pigmented cells. They will also alleviate alignment problems, allowing robust detection of CMC's in a clinical device. The ability to detect single CMC's makes this device a viable tool for monitoring metastatic melanoma cells in blood.

Melanin, a strong optical absorber within melanoma cells, absorbs low energy, rapid laser pulses thereby generating photoacoustic waves. The procedure for CMC detection would be performed by separating the white blood cells from a melanoma patient's blood sample. CMC's, if present, reside among the white blood cells. These cells are introduced into the photoacoustic detection system and are scanned for melanoma cells. In a healthy sample in which there are no melanoma cells, no acoustic pulses will be generated. In a sample containing metastatic melanoma, high frequency acoustic pulses would be generated and detected by the system.

This detection system manifests as two marketable products. The first product is the laser detection system described above that will be located at centralized testing centers, costing about $50,000-100,000. The second product is a CMC test kit costing approximately $50. The test kits would be used for regular monitoring of the 600,000 melanoma patients in the U.S. and by millions in the international markets. In this study, they will test up approximately 40 Stage IV melanoma patients during years 2 and 3. The actual number will depend on analysis of a pilot study currently underway. They will correlate disease state and survival using Kaplan-Meier plots to the number of CMC's detected in their blood samples. Using the results of this study, they will pursue FDA pre-market notification (510(k)) for the melanoma detector as a method to monitor disease state in advanced patients. This 510(k) strategy is conservative, with a high likelihood of being obtained. With subsequent funding from the SBIR and investor capital, they will conduct more efficacy studies to obtain 510(k) for detection of early metastasis.

Update: Two objectives were stated for this proposal: 1) Improve the photoacoustic detection system for robust detection of single CMC's, and 2) Perform a prospective clinical study correlating disease state and survival to numbers of CMC's detected in blood samples of Stage IV melanoma patients. The first year of this effort has been spent on the first objective and all of the facets of this objective have been performed successfully. The subsets of this objective and details of accomplishment are described below:

(1) The apparatus for optical detection of photoacoustic waves has been built and tested on melanoma cells from cell culture and from limited numbers of stage IV melanoma patients. The results of testing this device have been submitted to "Lasers in Surgery in Medicine" and "International Journal of Thermophysics". We have detected single cell events using this device in static and flow situations.
(2) We have developed an algorithm for automated wavelet denoising of the photoacoustic signals.

These signals are denoised online using a LabView interface from the detection electronics to a controlling laptop computer.

(3) In addition we have built and tested a two wavelength method using two tunable laser systems to statistically classify photoacoustic event as arising from hemoglobin or melanin. We are currently building a database of photoacoustic signals to improve statistical classification of all events tested in human samples in subsequent years. We have tested the system in accordance with the paradigm delineated in the research plan. In addition, we are conducting a blind study to test whole blood samples that have been spiked with low concentrations of melanoma cells along with control blood samples. We are planning to test 40 samples to determine sensitivity and specificty prior to enrolling human subjects next year.

In addition to the above progress, we have received notice that our patent claims have been accepted and we expect to receive a notice of allowance on our claims soon. The patent should then be issued early next year.

We have also been working with the Director of Entrepreneurship with the College of Engineering to obtain a round of funding from investors to form a management team for the company, Verapulse, to commercialize the technology. We have a pro forma document and business plan.

Project #:	09-1177
Project Title:	iPrep: Ophthalmic Povidone-Iodine Antiseptic Formulation
Award Amount:	$574,450
Center for Excellence:	Springfield
Lead Investigator:	Dr. Wendell Scott, St. Johns Medical Institute
Collaborators:	Dr. Paul Durham, Missouri State University

Summary:
Endophthalmitis is the inflammation of intraocular cavities of the eye most commonly caused by microbial infection, which can lead to decreased and permanent vision loss. Although the risk of endophthalmitis can never be completely eliminated, taking proper precautions such as pre- and post-surgical antisepsis, aseptic surgical technique, and prophylactic antibiotics can greatly reduce the incidence. An issue of particular concern involving ocular surgery is the best method for disinfection. The eye is highly sensitive to pH, tonicity, viscosity, concentration, and surface tension making it one of the most difficult parts of the body to disinfect.

Povidone-iodine (PVI) is currently used as a topical pre- and postoperative antiseptic for the eye through off-label methods of manually diluting the standard 10% product for the skin with sterile saline to achieve a 5% concentration. The off-label use of the 5% PVI causes local inflammation as well as death to the healthy epithelial cells that provide the covering of the eye. Since the current formulation is also highly acidic, it causes pain and discomfort to the patient when applied to the eye without first using an anesthetic. Their novel formulation of PVI, iPrep, has a neutral pH allowing for direct application to the eye without irritation and a lower concentration of iodine, which significantly reduces damage to healthy epithelial cells while maintaining its excellent antiseptic properties.

Other benefits of iPrep include the inability to develop bacterial resistance, capability to be used repeatedly in all age groups, inexpensive to manufacture, and highly effective against a wide range of infectious agents including Gram positive and negative bacteria, fungi, viruses, protozoa, and in particular, important ocular pathogens: Herpes simplex, adenovirus, HIV, and *Acanthamoeba*. Since the iodine concentration

has been optimized and the irritation factors have been eliminated, iPrep can be extended beyond pre- and post-surgical antisepsis to an over-the-counter eye antiseptic to reduce infections caused by minor cuts and scratches.

Another application of iPrep is for communicable ophthalmic infections such as Trachoma. Trachoma is a bacterial eye infection estimated to affect over 6 million people making it the leading cause of preventable blindness worldwide. Unfortunately, third-world countries are most affected by the debilitating effects of this disease. iPrep is a safe and inexpensive ophthalamic antiseptic that is anticipated to provide a readily accessible therapy which would drastically change the way Trachoma is treated.

The collaboration between St. John's Medical Research Institute and the Center for Biomedical and Life Sciences utilizes the strengths of a well established health care facility and those of higher education expertise to engineer and test iPrep both in animal and human trials. iPrep is ideal for investment by the MLSRB as a medical device according to the FDA United States Code [321] due to the straightforward nature of the transition to commercialization and the potential extensive impact to Springfield and the State of Missouri, as well as national and international communities.

Update: St. John's Medical Research Institute (SJMRI) and Dr. Wendell Scott have continued its progress to produce a novel formulation of povidone iodine specifically designed for use in ophthalmics. This enhanced formulation will allow ocular surgeons to place the product directly into the eye without causing irritation, reducing damage to healthy epithelial cells, and remaining highly effective as an antiseptic.

After thoroughly reviewing the product requirements for efficacy, toxicity, stability, osmolality, and iodine concentration, a formulation has been finalized. SJMRI will be submitting a provisional patent application and will continue stability and quality testing throughout the patent timeline.

Currently, SJMRI is drafting the pre-IND letter to submit to the FDA, which requests a formal meeting to discuss the project and to better understand the compliance and regulatory testing that must be in place to move forward. This step will also outline the clinical testing needed to prove effectiveness and address labeling requirements and batch tracking for production.

SJMRI will collaborate with local company Inveno Health to assist with commercialization efforts. SJMRI and Inveno have identified potential manufacturers and have begun discussions for feasibility testing and scale up production. Initial discussions around brand development and marketing strategy have begun and will continue to expand as the product progresses.

FY 2008 Life Sciences Research Trust Fund Grant Summary

Total Grant Funding Available in FY2008: $13,100,000

Research Funds Available in FY2008: $10,500,000
Commercialization Funds Available in FY2008: $ 2,600,000

Full Proposals Received -- Research

Centers for Excellence	Requested Research Funds	# of Requested Projects	Awarded Research Funds	# of Awarded Projects	% of Available Funds Awarded
Kansas City	$ 7,116,890	12	$ 2,650,915	5	25.2%
Springfield	$ 1,606,315	3	$ 950,908	2	9.1%
St. Louis	$ 4,210,849	1	$ 2,989,703	1	28.5%
Statewide	$12,962,501.33	18	$ 3,908,474	2	37.2%
Subtotals	**$25,896,555.33**	**34**	**$10,500,000**	**10**	**100%**

Full Proposals Received -- Commercialization

Centers for Excellence	Requested Commercialization Funds	# of Requested Projects	Awarded Commercialization Funds	# of Awarded Projects	% of Available Funds Awarded
Kansas City	$2,559,640	3	$ 325,000	1	12.5%
Springfield	$ 500,000	1	$ 0	0	0%
St. Louis	$1,326,775	1	$ 1,136,719	1	43.7%
Statewide	$4,586,459	4	$ 1,138,281	2	43.8%
Subtotals	**$8,972,874**	**9**	**$2,600,000**	**4**	**100%**

Summary of Grants Awarded in FY2008

Center for Excellence	Total Awards	Total Projects Awarded	Total % of Funds Available
Kansas City	$ 2,975,915	6	22.7%
Springfield	$ 950,908	2	7.3%
St. Louis	$ 4,126,422	2	31.5%
Statewide	$ 5,046,755	4	38.5%
Total	**$13,100,000**	**14**	**100%**

FY2008 Funding Summary – Research

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
13230	Ultrahigh-Throughput Sequence Profiling of Small RNA in Brachypodium Distachyon, an emerging model for Cereal and Biofuel Crops	Dr. Julia Chekanova	University of Missouri-Kansas City	Plant Science	$558,020
13234	Bone Fracture Repair in Animals Using a New Bond Cement	Dr. David Eick	University of Missouri-Kansas City	Animal Health	$786,998
13238	Evaluation of Candidate Diagnostic Targets for Johne's Disease in Livestock	Dr. Brian Geisbrecht	University of Missouri-Kansas City	Animal Health	$675,000
13243	Grape Polyphenols: Potential for New Commercial Products and Enhanced Plant Health	Dr. Laszlo Kovacs	Missouri State University	Gateway Fund	$897,955
13246	Novel Therapeutic Strategies for the Treatment of Eye Diseases in Animal	Dr. Ashim Mitra	University of Missouri-Kansas City	Animal Health	$312,273
13248	Integrated Program for the Development of Microalgae as Sustainable Resources for Biofuels and Biomaterials	Dr. Paul Nam	Missouri University of Science & Technology	Bioenergy	$526,906
13249	Insect-Deterrent and Antifeedant Properties of Ginkgo Biloba	Dr. Maciej Pszczolkowski	Missouri State University	Gateway Fund	$52,953
13250	Discovery and Utilization of Enzymes for Renewable Biofuels Production	Dr. Himadri Pakrasi	Washington University	Bioenergy	$2,989,703
13254	Identification of Functional Replication and Transcription Linked to Residues for Chromatin Assembly by Histone H3 Proteins in the Corn Smut Ustilago and the Yeast Saccharomyces	Dr. Jakob Waterborg	University of Missouri-Kansas City	Plant Science	$318,624
13321	Advancing Animal and Plant Agricultural Sciences in Missouri	Dr. Marc Linit	University of Missouri-Columbia	Animal, Plant, Environmental Science	$3,381,568
				TOTAL	**$10,500,000**

FY2008 Research Project Summaries

Project #:	13230-2007
Project Title:	Ultrahigh-Throughput Sequence Profiling of Small RNA in Brachypodium Distachyon, an Emerging Model for Cereal and Biofuel Crops
Award Amount:	$558,020
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Julia Chekanova, University of Missouri-Kansas City
Collaborators:	Todd Mockler, Oregon State University

Summary:
Progress in understanding the basic biology and mechanisms of gene function in monocot grasses (the world's predominant grass species), including cereal species cultivated for food and feed, as well as dedicated biofuel crops such as switchgrass, has been severely constrained for many years due to the lack of convenient experimental systems (i.e. model crops). For example, RNA has emerged during the past decade as a key controller of genome function, yet very little information on small RNA function in monocot species is currently available. Brachypodium distachyon (a genetic relative of wheat, barley, and switchgrass) has recently emerged as a premier model for studying how genes function in more genetically complex temperate grasses because of its simple growth requirements, rapid life cycle, small size, and relatively simple "genome," or catalogue of hereditary information. This study will use the model grass, Brachypodium, to fill the significant gap in knowledge that exists for monocot grasses such as wheat, barley, oats, and switchgrass. Ultimately, this new knowledge will benefit Missouri's agriculture and biofuels industry through higher yields for these crops and more efficient energy conversion.

In the first years of funding small RNAs have been firmly established as key regulators of genome function in diverse organisms including plants, yet the biology of small RNAs in monocot plants remains poorly studied. The goal of this proposal is to create a comprehensive catalog of small regulatory RNAs in Brachypodium distachyon, the emerging model system for such agriculturally important species of cereal crops as wheat, corn, rice, barley, as well as for dedicated biofuel crops such as switchgrass, i.e. for the most economically important group of plants. Because small RNA spectra are known to differ between different tissues, stages and growth conditions, they chose to focus this year on leaf, root and stem tissues, as well as on leaves of plants infected with the plant pathogen fungus Magnaporthe grisea. Magnaporthe grisea is the causal agent of rice blast disease, which is the source of tremendous crop losses worldwide.

They have obtained and propagated Brachypodium distachyon line Bd21, established collaboration with the US Fungal Genetics Stock Center that is housed at UMKC, and obtained USDA permit to carry out plant infections with pathogenic fungi. They identified two strains of Magnaporthe grisea (Guy-11 and 70-15) causing infection of Brachypodium distachyon line B21 plants and carried out pilot as well as full scale plant infections. Small RNAs were isolated from three tissue types (leaves, roots, stems) as well as from leaves of plants infected with Magnaporthe grisea. These small RNAs are being used for small RNAs library construction. Solexa deep sequencing of first six small RNA libraries is scheduled for January 2009.

Update: The goal of this project is to identify the majority of small RNAs in Brachypodium, including those that are cereal-specific and those regulated by abiotic and biotic stress, as well as those that are induced in response to infection by pathogenic fungi.

Small RNAs isolated from total plant and tissue types such as leaves, roots and stems had been used for small RNAs library construction and Solexa deep sequencing. We have also sequenced small RNAs

isolated from leaves of plants infected with the plant pathogene fungus Magnaporthe grisea, a causal agent of rice blast disease. Leaves of plants subjected to mock infection were used as a control. In order to be able to subtract Magnaporthe specific small RNAs from infection induced Brachypodium small RNAs we had also sequenced small RNA population from appressoria (fungi hyphal branch which facilitates penetration of the host plant).

Salt, drought and cold stress are among the most frequent and devastating challenges that affect agriculturally important crops. In order to elucidate small RNA-regulated gene circuits under most common stress conditions that crop plants encounter we subjected plants to numerous aboitic and biotic stresses such as heat, cold, drought and salt. Leaves and roots of plants challenged for a different period of time with either heat or cold were used for small RNA library construction, all these libraries had been deep sequenced. We are in a process of collecting tissues from plants subjected to various drought and salt stresses. Analysis of sequenced small RNAs had being initiated.

Project #:	13234-2007
Project Title:	Bone Fracture Repair in Animals Using a New Bond Cement
Award Amount:	$786,998
Center for Excellence:	Kansas City
Lead Investigator:	Dr. David J. Eick, University of Missouri-Kansas City
Collaborators:	Donna M. Pacicca, The Children's Mercy Hospital

Summary:
Pets and large animals, such as horses, currently benefit from biomaterials (materials used in medical devices that interact with the body) that are used to stabilize and heal bone fractures. Frequently, a bone cement is used to stabilize fractures and prosthetic devices such as those used for hip replacement in dogs with hip dysplasia, a disease that can cause crippling lameness and painful arthritis. The bone cement that is currently used for this purpose is a strong resin (called PMMA) that has several significant drawbacks – it impedes the healing of the bone due to severe toxicity and heat generation and it contracts while solidifying.

The scientists leading this project have developed a silorane-based resin that is superior to PMMA in several important ways. This resin, for example, maintains the same strength as PMMA without contracting or shrinking while drying. It is also less toxic and generates less heat. Additionally, preliminary data suggest this resin actually supports, rather than hinders, bone formation. The results of this study will be the development of composites that have enhanced strength and compatibility with the body. Fillers will be included in the form of hollow microspheres (biodegradable glass) that could be used to contain and act as carriers for antibiotics or growth factors that induce bone growth and blood vessel formation. Accomplishment of these goals will lead to a commercial application that will improve bone health in both large and small animals.

The broad, long-term objective of this grant application was to develop drug delivery strategies to improve ocular absorption of topically applied veterinary drugs such as erythromycin, prednisolone, acyclovir (ACV) and bimatoprost for the treatment of bacterial keratitis, inflammations and viral corneal keratitis respectively. They have already reported to The Missouri Life Sciences Research Board that erythromycin and bimatoprost have been replaced with ganciclovir (GCV) and gatifloxacin respectively due to higher clinical significance to veterinary health sciences. The first specific aim involves the synthesis and characterization of the sterioisomeric prodrugs of ACV, GCV and prednisolone. This goal has been

completed. The prodrugs have been characterized for their interaction with peptide transporters. Bioreversion and stability studies for ACV and GCV prodrugs have been carried out.

They have screened gatifloxacin for its affinity towards the efflux transporters (P-glycoprotein, Multidrug resistance associated protein and Breast cancer resistance protein). Synthesis of sterioisomeric prodrugs of gatifloxacin is in progress. They have also prepared and characterized nanoparticles of ACV and its prodrugs. They have synthesized and characterized a novel pentablock (PB) polymeric material made of multipolymer blocks like polyglycolide, polyethylene glycol and polycaprolactone (every block of PB polymer is FDA approved for human use). Since polyglycolide has a faster biodegradation profile relative to polycaprolactone, varying the ratios of these two blocks polymers in the PB polymers can optimize the long term release of these sterioisomeric prodrugs in the eye.

They are planning to utilize this novel PB polymer (US patent in preparation) to prepare and characterize nanoparticles. Prodrug entrapment efficiency, particle size, and release profiles will be optimized by adjusting polymeric block ratios of PB polymer. Nano particles containing ACV, GCV, prednisolone, gatifloxacin and their prodrugs will be ready for evaluation in the rabbit eye in next two months. A suitable long term delivery system will be available for testing in animals (Cats and Dogs) by the end of 2009.

Update: We propose that materials can be generated that will not only stabilize the fracture and be biocompatible, but will enhance fracture repair and the osseointegration of prosthetic devices.

Specific Aims
1: Develop and synthesize resins, fillers, and/or composites suitable for testing in bone stabilization models. The synthesis of the two components of Sil-Mix was determined. Previously a light-activated initiation system was developed for the Sil-Mix resin. Chemical activation is preferred for the application of a bone stabilizer. Currently a biocompatible chemical activation system has not been identified. While this search continues, a mixed initiation system using both light and chemical initiation has been identified for preliminary testing. Surface modifications using 2-(3,4-Epoxycyclohexyl) ethyl trimethoxysilane (ECHE-TMS) have been developed for use with the alumina nanorods and glass filler particles and have been shown to enhance the resin-matrix interface.

2: Characterize the physical and mechanical properties of the newly developed bone cements/stabilizers. Orthopedic surgeons have been included in discussions to determine the handling characteristics desired for a bone stabilizing material. A 'bone stabilizer' has been defined as a material that is easily transportable, has an exotherm below 45°C, cures within 15 minutes while allowing ample working time and once cured prevents both torsion and bending movements of the stabilized bone. Appropriate tests have been identified and developed to characterize the strength, elastic modulus, fracture toughness, viscosity, cure time, dough time, working time and exotherm. A mixture design has been developed to optimize the filler combination with respect to these characteristics. Preliminary tests have shown that the addition of fillers increases the stiffness (modulus) of the material but results in a slight decrease in strength.

3: Determine the effects of the newly developed bone stabilizers on biological response of bone cells and effects on bone tissue healing, formation and osteointegration. Studies were performed comparing the cytotoxicity of MLO-A5 cells with the three available initiation systems (photo, mixed, chemical) for the silorane. Results showed that both the photoinitiation and mixed initiation system had low cytotoxicity, similar to cells grown on standard cell culture plastic. However, the purely chemical initiation system resulted in severe cytotoxicity. This was potentially due to the low pH of this material due to the strong

acids used as initiators. It was also determined through these studies that cells do not tend to adhere to the silorane material, but instead form viable clumps of cells. Animal studies have been developed to determine the ability of filled Sil-Mix to stabilize bone. Pilot testing have been completed on extracted mouse femurs with a clean-cut fracture introduced with a circular saw. Photoinitiated or mixed initiation silorane was applied around the bone creating a ~1-mm wide by 1-2 mm thick band of stabilizing material. From these preliminary tests it was shown that the slower curing time of the mixed initiation system impedes the proper placement of the stabilizing material. Current work is focused on the development of a more viscous material to improve control during placement of the material. These preliminary studies have shown that the stabilized bones with current materials achieve approximately 60% of the flexural strength of unfractured bones. The incorporation of filler particles and improved initiation systems will likely improve the flexural strength of the stabilized bones.

Project #	13238-2007
Project Title:	Evaluation of Candidate Diagnostic Targets for Johne's Disease in Livestock
Award Amount:	$675,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Brian V. Geisbrecht, University of Missouri-Kansas City
Collaborators:	John P. Bannantine, National Animal Disease Center, U.S. Department of Agriculture

Summary:
Johne's disease is a fatal livestock disease that results from a chronic infection of the gut (stomach and intestines) by the widely distributed environmental organism Mycobacterium avium paratuberculosis (MAP). The economic impact of Johne's disease accounts for an estimated loss of $1.5 billion per year to U.S. livestock producers. Johne's disease is widely considered to be one of the most serious issues affecting livestock in Missouri.

In addition to livestock concerns, mounting evidence suggests that milk production from infected animal results in contamination of a large percentage of the nation's dairy supply, since common methods of pasteurization do not destroy MAP. It is therefore essential for both economic and human health related reasons to have adequate methods of diagnosing Johne's disease in cattle as well as methods for testing dairy products for MAP contamination. Unfortunately, affordable and effective tests for this organism and Johne's disease have yet to be developed.

This study will examine target immunological biomarkers (fragments of DNA sequence that cause disease) derived from the MAP bacterium and assess the feasibility of translating these protein antigens (molecules that stimulate an immune response) into affordable diagnostics for MAP infection in livestock and their agricultural products.

Update: The vast majority of the MAP gene products have no known function, and little is known about how this pathogen prevents a sterilizing response by the host during its long, latent period between the initial infection, which occurs shortly after birth, and the final, fatal stage, which could occur after a decade. Of the approximately 4300 genes encoded in the MAP genome, an expanding set of approximately 50 individual proteins have been selected based on predicted secretion or expression on the surface of the pathogen. These proteins are most likely to elicit a specific host response, and are also most interesting from a pathobiology perspective because they might be involved in modulation of the host response to infection. Several of these proteins have been shown to elicit a very strong, possibly specific antibody response in some infected cattle. We have been quite fortunate to receive a small collection of bovine sera from infected animals which can be used to assess our progress in designing a diagnostic test.

Rather than using a crude mixture of total MAP proteins, which is susceptible to a great deal of batch to batch variability, we are focusing on highly defined, well characterized preparations of purified proteins. Using a highly efficient pipeline to process and analyze these proteins in parallel, we have successfully produced 30 of these proteins in a bacterial expression system. To date, four of these proteins have been crystallized, and the structures of three, (MAP1204, MAP1272c, and MAP2168c) have been determined at atomic resolution. Two of these proteins are members of the P60 family of proteins, which are critical for separation of daughter cells during bacterial division. Based on these structures, an expanded analysis of the MAP genome has revealed three additional members of the P60 family, which have been added to the set of genes being analyzed. It is not unusual for a bacterial species to express several different P60 proteins. These MAP proteins are highly homologous to each other, however, confounding the issue of host recognition specificity. Two monoclonal antibodies against MAP1272c have been generated in mice by John Bannantine, our collaborator at the USDA-ARS. Although the immunogen used was MAP1272c, we are exploring the cross reactivity of these enzymes with similar portions of the four other MAP P60 proteins. Monoclonal antibodies can be used in conjunction with our recombinant proteins to develop rapid colorometric diagnostics, similar to early pregnancy or toxicology sticks. Instead of urine, a small sample of total bovine serum would be used to detect anti-MAP antibodies.

Additionally, the structure of a protease stable fragment of the MAP2168c ectodomain has been solved. This protein has no assigned function as yet, however our structure has induced us to explore its role in MAP biology. In addition to another highly similar protein in the MAP genome, there are closely related proteins in several other human pathogenic *Mycobacteria*, including the causative agents of tuberculosis and Hansen's disease, as well as two pathogenic *Corynebacteria* species. Further analysis of the MAP genes surrounding the 2168c locus suggests it is involved in the synthesis of non-ribosomally decoded peptides. These peptides have various functions, including iron uptake from the environment. Iron ions are essential for metabolism and structure stabilization, and it has been shown that abrogation of iron uptake leads to bacterial death. Iron uptake in mammals uses a distinct mechanism; therefore understanding how MAP accomplishes this task at the molecular level furthers our knowledge of this pathogen's biology.

Project #:	13243-2007
Project Title:	Grape Polyphenols: Potential for New Commercial Products and Enhanced Plant Health
Award Amount:	$897,955
Center for Excellence:	Springfield
Lead Investigator:	Dr. Laszlo Kovacs, Missouri State University
Collaborators:	Wenping Qiu, Missouri State University; Richard Biagioni, Missouri State University; Paul Durham, Missouri State University; Daniel Schachtman, Donald Danforth Plant Science Center; and Oliver Yu, Donald Danforth Plant Science Center

Summary:

Grapes synthesize a plethora of polyphenolic compounds (such as tannins and lignins), many of which improve the health of both the plant and the human who consumes it or its product (e.g. wine or juice). This study will focus on two varieties, Norton and Cabernet Sauvignon, the former of which is the most prominent wine grape in Missouri. The project scientists will work to identify the individual compounds, or classes of compounds, that provide the health benefits provided by grapes and grape products. The resulting information will lead to the development of novel high-value grape products, such as wines, food supplements, and herbal condiments with scientifically-proven dietary value. This research project will also identify genes that direct polyphenol synthesis in the berries that respond most effectively to plant

pathogens or diseases. Knowledge acquired from these studies will lead to healthier fruit products and hardier plants, resulting in improved human health and lower fungicide usage in Missouri vineyards.

In the first year of funding they collected berry tissue (seed and skin) samples from two different grapevine varieties (Norton and Cabernet Sauvignon) at defined developmental stages all through the growing season. They are extracting nucleic acids from these tissues to begin studying genes of enzymes that synthesize polyphenolic compounds in the berry. In addition, they began collecting information on the key regulators of the flavonoid pathway, the route by which the major polyphenolic compounds are synthesized in grape cells. Twelve genes of these regulators have also been cloned and are now being introduced into grape roots to determine how they influence flavonoid biosynthesis.

A comparative analysis of the polyphenolic compounds themselves is underway in both Norton and Cabernet Sauvignon. More than ten novel anthocyanins (color-producing polyphenols) that are specific to Norton were already identified at the level of molecular structure for the first time. Chromatographic studies also are underway to compare the amounts of seed polyphenols as they accumulate during the course of berry development. In bioactivity studies, they have found that polyphenols extracted from the seed possess considerably higher anti-inflammatory activity than those from the berry skin. Seeds also contain higher quantities of these compounds, with maximal quantities measured at the developmental stage at which the berry starts to change color and ripen (veraison).

Currently, large-scale experiments are underway in which seed extracts are fractionated and the anti-inflammatory activity of the various fractions are tested. Work is also in progress to determine how long the polymers are that accumulate in the seed and in the berry skin, with results already showing that proanthcyanidins form shorter polymers in the seed than in the berry skin. Polymer length may be one of the characteristics that are important in determining the anti-inflammatory potential on these compounds.

Update: In part of the project that targeted the identification of pharmacologically active substances in grape berry tissues, we completed all the laboratory analysis (various extractions from seeds and berry skin plus the *in vitro* and *in vivo* bioassays of the resulting extracts) that were planned, except for the work on the polymer length of proanthocyanidins. This work encountered some difficulties earlier and was delayed, but is progressing well now. We are also following up on our new findings, most notably, on the novel class of biologically active substances that we identified in grape seeds. We demonstrated that these methanol-extractable compounds have anti-inflammatory activity in mammalian cells both *in vivo* and *in vitro*. This discovery was made possible by our bioactivity-driven approach in which each step of our investigation was guided by measurements of anti-inflammatory capacity in a mammalian cell-based bioassay. Commercialization of this substance has both nutraceutical and agricultural potentials, as there is increasing interest in the incorporation of plant-derived health-promoting substances in the human diet. These discoveries are also significant scientifically, as they help explain the pharmacological properties of plant-derived substances.

In the plant metabolite biosynthesis part of the project, we have completed all chromatographic work on the polyphenolics and plant hormones, as well as gene expression data analysis in the developing seeds. We are currently in the process of completing microarray data analysis in the berry skin. The results show that certain proanthocynidins and other bioactive substances are present at their highest levels at around veraison. At veraison, there are major changes taking place in seed development as the seeds began to prepare for dormancy. This observation is supported by the changes in the levels of plant hormone levels and hormone-related gene expression. We have plans to further explore these phenomena in the future. We also plan to identify other berries that contain similar bioactive substances. Wild or native plants in

Missouri may have elevated levels of these compounds, as seed extracts from of the Missouri grape variety Norton has apparently higher biological activity than those form the world-variety Cabernet Sauvignon. Moreover, we identified ten polyphenolic compounds that were present in Norton, but were undetectable in Cabernet. The differences in polyphenolics were paralleled by clear-cut differences in the expression of genes that regulate secondary metabolism. It requires further investigations to determine if there is a cause-effect relationship between the variation in gene expression and the variation in the levels of secondary metabolites and the strong plant disease resistance in Norton.

Project #:	13246-2007
Project Title:	Novel Therapeutic Strategies for the Treatment of Eye Diseases in Animal
Award Amount:	$312,273
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Ashim K. Mitra, University of Missouri-Kansas City

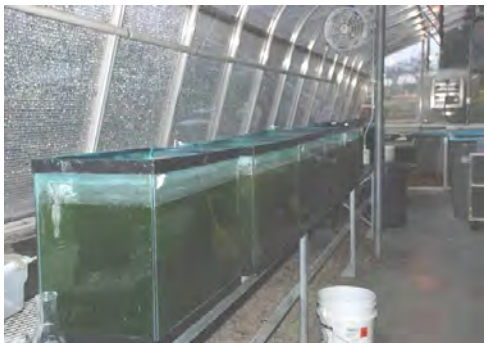
Summary:
Topical administration is the most preferred and convenient route for treatment of veterinary eye diseases. However, drug levels absorbed by the eye for most topically applied drugs are less than one percent of the applied dose. Obviously this is an inefficient treatment method. The objective of this research project is to develop drug delivery strategies to significantly improve eye absorption of topically applied veterinary drugs such as erythromycin, prednisolone, acyclovir and bimatoprost for the treatment of bacterial keratitis, inflammations, viral corneal keratitis, and glaucoma. With these grant funds, scientists will continue research on a drug that, when added to eye medicine, results in greater solubility and eye absorption. In addition, a drug is being tested that uses microscopic particles suspended in a gel solution to increase the time the substance resides on the eye and sustains the release of the drug. This strategy allows a single treatment application for one week therapy, causing much higher efficacy of the applied medication, particularly in companion animals.

Update: The broad, long-term objective of this grant application was to develop drug delivery strategies to improve ocular absorption of topically applied veterinary drugs such as erythromycin, prednisolone, acyclovir (ACV) and bimatoprost for the treatment of bacterial keratitis, inflammations and viral corneal keratitis respectively. We have already reported to the MLSRB that erythromycin and bimatoprost have been replaced with ganciclovir (GCV) and gatifloxacin respectively due to higher clinical significance to veterinary health sciences. The first specific aim involves the synthesis and characterization of the sterioisomeric prodrugs of ACV, GCV and prednisolone. This goal has been completed. The prodrugs have been characterized for their interaction with peptide transporters. Bioreversion and stability studies for ACV and GCV prodrugs have been carried out. The pharmacokinetic parameters of these prodrugs have also been studied in ocular tissues. We have screened gatifloxacin for its affinity towards the efflux transporters (P-glycoprotein, Multidrug resistance associated protein and Breast cancer resistance protein). Synthesis of sterioisomeric prodrugs of gatifloxacin is in progress. We have also prepared and characterized nanoparticles of ACV, GCV and Prednisolone along with their prodrugs and generated release profiles. The prodrugs of prednisolone suffer from poor entrapment efficiency in nanoparticles hence we are using prodrugs of dexamethasone as model substrates to solve this problem. We are using a unique hydrophobic ion pairing with complex technique to enhance the entrapment efficiency of these prodrugs and the results from these studies will be applied to prednisolone prodrugs. We have synthesized and characterized a novel pentablock (PB) polymeric material made of multipolymer blocks like polyglycolide, polyethylene glycol and polycaprolactone (every block of PB polymer is FDA approved for human use). Since polyglycolide has a faster biodegradation profile relative to polycaprolactone, varying the ratios of these two blocks polymers in the PB polymers can optimize the long term release of these sterioisomeric prodrugs in

the eye. We are planning also planning to utilize this novel PB polymer (US patent in preparation) to prepare and characterize nanoparticles. Prodrug entrapment efficiency, particle size, and release profiles will be optimized by adjusting polymeric block ratios of PB polymer. Nano particles containing ACV, GCV, prednisolone, gatifloxacin and their prodrugs will be soon be ready for testing in rabbits. A suitable long term delivery system will be available for testing in animals (Cats and Dogs) by mid 2010.

The funds allotted by the State of Missouri for this grant proposal have been used to acquire a high performance liquid chromatography system along with various cell culture material and reagents required to accomplish the tasks proposed in the project.

Project #:	13248-2007
Project Title:	Integrated Program for the Development of Microalgae as Sustainable Resources for Biofuels and Biomaterials
Award Amount:	$526,906
Center for Excellence:	Statewide
Lead Investigator:	Dr. Paul Nam, Missouri University of Science and Technology
Collaborators: Keesoo Lee, Lincoln University; Virgil Flanigan, Missouri University of Science and Technology; and Fabio Rindi, University of Alabama	



Summary:
Scientists from multiple disciplines and institutions will work collaboratively to develop algae as a potential solution to growing energy and environmental challenges. This project seeks to: develop sustainable and less expensive methods for the capture and conversion of solar energy in algae; develop methods to use algae biomass as a renewable fuel source; and develop practical and environmentally responsible methods of carbon dioxide capture and sequestration. The major thrust of the research program will focus on the following: 1) identification of high yielding, hardy, pest resistant algae strains; 2) developing economically viable, commercial scale algae cultivation systems; 3) identifying an effective system for extracting oil from wet algae for conversion into biodiesel; and 4) testing methods for fermenting algae carbohydrates into ethanol. Integration of these innovations should yield a comprehensive algae cultivating and refining system that can economically mass-produce biomass feedstock for conversion into biofuels, biopolymers (plastics), and other valuable products.

Update: The multidisciplinary algae research and experiential-education programs at Missouri University of Science & Technology and the Lincoln University campuses were expanded further with the additional financial support from the USDA-CSREES. More than 300 native species of microalgae were isolated from natural water bodies in the Midwestern United States and screened for the ultimate goal of mass cultivating in Missouri and the surrounding states, and for their potential as biomass and biodiesel sources. All of the isolates were categorized based on the morphological appearance of the culture and the microscopic cellular appearance of the isolated colonies. Lipid contents were determined for selected strains that demonstrated relatively quick growth. One strain (*Scenedesmus* sp.) which demonstrated the higher growth rate, resistance to invasion, and sufficient lipid content has been investigated for its potential as a sustainable source of biodiesel feedstock that can be produced in the central Missouri region. A pilot 10,000-gallon open-pond algae cultivation system that can utilize the flue gas carbon dioxide was

constructed at the Central Electric Power Cooperative power plant in Chamois, Missouri for the demonstration of large-scale cultivation and harvesting processes. For the efficient production of biodiesel from oil-bearing crops including microalgae, the catalyst-free transesterification reaction using supercritical methanol was developed. Improved methods for pretreatment and hydrolysis of microalgae as cellulosic ethanol feedstock are also being investigated.

Project #:	13249-2007
Project Title:	Insect-Deterrent and Antifeedant Properties of Ginkgo Biloba
Award Amount:	$52,953
Center for Excellence:	Springfield
Lead Investigator:	Dr. Maciej Pszczolkowski, Missouri State University

Summary:
The goal of this research project is to develop a novel pest control strategy targeting internal fruit feeding insects. Codling moth, the most significant apple pest problem for Missouri growers, infests the fruit as "neonate," or newborn, larvae within twelve hours after hatching from the egg and stays inside the fruit until its development is complete. Recently advocated Codling moth control measures that target adults are only at an early stage of development in Missouri and may not be effective enough to provide satisfactory control measures. Historically, broad-spectrum contact insecticides have been used to target larvae just after hatch. However, these insecticides pose a severe risk to human health, negatively impact the natural environment, and will soon be banned by the federal "Food Quality and Protection Act." If no alternatives are commercially available after this pesticide is banned, it is estimated that Missouri apple growers will lose at least $5.2 million in annual income.

It is already known that ethanolic extracts from Ginko biloba (a Chinese tree that is exceptionally resistant to insect pests) prevents apple infestation by Codling moth neonates. This study will determine which Ginko substances discourage neonates from infesting fruit. By identifying these substances, the study will open a new avenue for Codling moth control with substances derived from an herb which has been known to be beneficial to human health, thereby helping to reduce potential human health risks and minimize adverse environmental effects from currently used Codling moth control measures. The study will be undertaken in cooperation with an internationally recognized expert on modifying Codling moth larvae behavior with plant extracts. The results will be licensed to a Missouri-based company which produces chemicals for agricultural applications.

This two-year project aims to identify the components of Ginkgo biloba that have antifeedant and deterrent properties toward the major cosmopolitan pest of apples, codling moth, Cydia pomonella.

During the first year, the PI and co-workers established a season-independent choice assay for testing Ginkgo extracts. Methodology of this assay has been published in a peer-reviewed research paper, which

is scheduled to appear in Journal of Entomological Society of British Columbia by the end of December 2008. Moreover, they partitioned Ginkgo extracts into five fractions, each containing different class of chemicals that are found in Ginkgo foliage. Three classes of Ginkgo constituents were inactive in their assays. One class is still being tested. Yet another class of Ginkgo constituents has deterrent properties and prevents fruit infestation by codling moth larvae in dose- dependent manner. They are in the process of identifying the deterrent chemical compound in this fraction. Their results pave the way to implementation of strategies of codling moth control with plant derivatives, so called secondary metabolites. These may be synthesized on a larger scale and used for sprays against codling moth larvae. Alternatively, their chemistry may suggest how codling moth- resistant apple varieties could be rationally designed.

Update: The project led to the identification of three candidate Ginkgo-derived chemicals that have deterrent activity against apple pest, the codling moth, activity of which has not been reported previously. At the beginning of the project we anticipated only one such a compound. The plan is to patent the use of the three candidate Ginkgo-derived chemicals against codling moth and license the results to Missouri-based plant protection companies. We are currently assembling data and performing final experiments for two major publications in the area of entomological science.

Project #:	13250-2007
Project Title:	Discovery and Utilization of Enzymes for Renewable Biofuels Production
Award Amount:	$2,989,703
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Himadri Pakrasi, Washington University
Collaborators: Largus (Lars) Angenent, Washington University; Rajeev (Reggie) Aurora, St. Louis University; Richard Axelbaum, Washington University; Roger N. Beachy, Donald Danforth Plant Science Center; Pratim Biswas, Washington University; Robert Blankenship, Washington University; Jeffrey I. Gordon, Washington University; Tuan-Hua David Ho, Washington University; Monty Kerley, University of Missouri-Columbia; Shelley Minteer, Saint Louis University; Ralph Quatrano, Washington University; Monica Schmidt, Donald Danforth Plant Science Center;Thomas Smith, Donald Danforth Plant Science Center; Gary Stacey, University of Missouri-Columbia;Teresa Thiel, University of Missouri-St. Louis; Xuemin (Sam) Wang, University of Missouri-St. Louis; Dong Xu, University of Missouri Columbia; Oliver Yu, Donald Danforth Plant Science Center; and Zhanyuan Zhang, University of Missouri- Columbia	

Summary:

This proposal includes a comprehensive set of biofuels related research projects by members of the Missouri Biofuel Research Consortium in St. Louis, a group of 20 world class plant scientists. The projects focus on three areas: improving the efficiency of transforming biological materials into energy, enhancing the reliability and cost effectiveness of biofuels, and increasing the efficiency of transforming sunlight into energy via biological materials (plants and algae). While fossil fuels will remain a critical fuel for energy generation in the foreseeable future, bio-derived fuels will be an important component of our regional and national energy portfolio. Availability of cheap, abundant energy is imperative for the economic prosperity and national security of any country. This proposal will help biofuels transition from a boutique to a primary energy source, improving both of these important ends.

Update:

ALGAE GROUP

• Biodiesel is an alternative transportation fuel that is currently made from vegetable oils by transesterification using methanol or ethanol, which are derived from fossil fuels. Biodiesel, or methyl- and ethyl- esters of fatty acids, has recently been synthesized in *E. coli* by the expression of three genes.

Investigators at the *University of Missouri – St. Louis* have modified the three genes needed for FAEE (fatty acid ethyl esters) production so that the genes necessary for biodiesel production are expressed under the control of the strong inducible *nifH* promoter. This promoter functions well in specific cells in cyanobacteria called heterocysts, and already produce long chain lipids that might serve as a substrate for production of biodiesel.

• To identify cellular factors that enhance oil and biomass production, investigators at the *University of Missouri – St. Louis* have also been identifying genes that regulate storage lipid synthesis and accumulation. Several candidate genes have been found to be involved in storage oil accumulation in seeds. Investigators are using *Camelina sativa* as a model to test the function of these genes in oil production. Camelina is an emerging new oil crop that requires low input of nutrients and water, has a short life cycle (90 days from seeds to seeds), and is easily transformed. Two genes, a Myb transcriptional factor and a phospholipase D, have been placed under the control of a seed specificpromoter.

• Transgenic plants harboring the gene of interest have been obtained, as confirmed by PCR and immunoblotting.

• Using Trust Fund funds, a GC-MS (Gas chromatography-mass spectrometry) facility has been established at *Washington University* for metabolite concentration and isotopomer distribution measurement. Investigators have characterized *Thermoanaerobacter sp. strain X514* and discovered a novel enzyme which can be utilized for butanol synthesis. Meanwhile, the impacts of carbon and nutrient sources on the CO2 fixation in *Roseobacter denitrificans* OCh114 has been examined, which reveals a unique CO2 fixation pathway without using Calvin Cycle.

• The LSTF funds have been used by investigators at *Washington University* for the development of an algal system that produces high level of biohydrogen, a clean source of energy. In this system, sunlight and CO2 are directly used to first capture carbon in the form of glycogen, which is then, in turn, converted inside the cell factory to hydrogen.

ENZYME GROUP

• A central challenge for the production of biofuel using lignocellulose is to identify enzymes, cellulases, that can efficiently break down the cellulose into sugars that can be easily used by an organism. However, no simple inexpensive assays currently exist that can be used for identifying, screening and optimizing cellulases that can digest celluloses from different sources (e.g. Corn,hardwoods, grasses etc.). To that end, investigators at *Saint Louis University* have developed a sensitive, high throughput (96 or 384 well plates), robust, and rapid (30 minutes) fluorescence assay for cellulases that uses the dye Congo red. A manuscript describing this assay, and a patent for the assay are currently being prepared.

• Today, butanol is primarily used as an industrial solvent, however, butanol can also be used as a fuel in present transportation and as a replacement for the use of gasoline and ethanol. To eliminate the problems associated with butanol toxicity to the microbes that can be used to produce butanol and specific product production, *Saint Louis University* investigators are proposing an enzymatic bioreactor by which pyruvate intermediates will be enzymatically converted to butanol. By varying the growth conditions along with cell lysis protocols, investigators were able to increase the NADHdependent butanol dehydrogenase activity in crude extract by 605.3 fold. *Washington University* investigators have confirmed that two inducible promoters are available for use for tissue growth of moss. A secretion sequence will be tested and confirmed in a working sixliter bioreactor in a designated lighted growth chamber for biomass accumulation. Methods to improve production of transgenic protein have been identified, and production has increase by at least four-fold over the last six months. Lab members are presently amplifying tissue to check protein accumulation and evaluate the specific activity of these enzymes for use in biomass degradation

• In order to reduce lignin levels during or before full maturation, investigators at the *Danforth Plant Science Center* has generated transgenic Arabidopsis plants containing genes that confer constitutive or inducible silencing of genes that play a key role in lignin biosynthesis. Results suggest that it will be

possible to alter lignocellulose composition in some plants without affecting normal growth and development.

• Investigators at the *University of Missouri – Columbia* have been working to identify enzymes capable of degrading plant cell wall polysaccharides into fermentable sugars. It is apparent that for this to be economically feasible, such enzymes should be produced in the cellulosic feedstocks themselves. In theory, such enzymes could be introduced into cellulosic feedstock prior to the pretreatment that is required. However, since pretreatment typically involves elevated temperature and a range of pH, thermostable and pH-stable enzymes are required. The alternative that is being proposed and examined is to produce (in feedstock) the cell wall-degrading enzymes, and then to integrate the enzymes into existing production practices, downstream of the pretreatment step.

• Also at the *University of Missouri – Columbia*, investigators have further developed MUFOLD, a software package, for predicting tertiary structure from a protein sequence. In order to improve the efficiency of bioenergy, it is often important to characterize and design related genes through their protein structures. MUFOLD demonstrated proof of principles in the 2008 community-wide experiment for protein structure prediction (CASP8), and was ranked top 4 in the template-free prediction category among 81 teams.

• Studies at the *Danforth Plant Science Center* are also elucidating how the enzyme SusG works with other membrane components to break down and import starch for fermentation. SusG is an a-amylase and part of a large protein complex on the outer surface of the bacterial cell and plays a major role in carbohydrate acquisition by the animal gut microbiota.

Project #:	13254-2007
Project Title:	Identification of Functional Replication and Transcription Linked to Residues for Chromatin Assembly by Histone H3 Proteins in the Corn Smut Ustilago and the Yeast Saccharomyces
Award Amount:	$318,624
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Jakob H. Waterborg, University of Missouri-Kansas City

Summary:

Fungi are often serious plant pathogens which threaten and diminish agricultural crops. In order to control fungi related diseases better, and to learn how one can directly interfere with or change fungal actions, control of gene expression must be better understood. This study uses Corn Smut, a fungal disease that infects corn, as a model. It seeks to understand the role specific histones (spools around which DNA winds) play in growth and development of the fungus. This basic research could eventually lead to applications to guard corn and other important cash crops from fungal diseases.

Update:

- Identification that newly synthesized histone H3 in fungi, including smut and yeast, has a unique and characteristic post-synthetic modification pattern that allows separation from mature H3, has provided a new and unanticipated tool for the study of chromatin assembly. HPLC and Mass-Spectrometry is being used for full characterization.
- Homologous gene replacement of distinct histone H3 forms and their promoters has been accomplished and is being optimized. The modified endogenous H3 histone proteins created are a tool that was identified as one of the specific aims of the funded project.

Project #:	13321-2007
Project Title:	Advancing Animal and Plant Agricultural Sciences in Missouri
Award Amount:	$3,381,568
Center for Excellence:	Statewide
Lead Investigator:	Dr. Marc J. Linit, University of Missouri-Columbia
Collaborators: Rod Geisert, University of Missouri-Columbia; Jack Jones, University of Missouri-Columbia; Rob Kallenback, University of Missouri-Columbia; Monty Kerley, University of Missouri-Columbia; Scott Peck, University of Missouri-Columbia; Keith Striegler, University of Missouri-Columbia; Jinglu Tan, University of Missouri-Columbia; Jerry Taylor, University of Missouri-Columbia; Jay Thelen, University of Missouri-Columbia; John Walker, University of Missouri-Columbia; Wenping Qiu, Missouri State University, Mountain Grove	

Summary:
This University of Missouri proposal focuses on strategic investments across a broad spectrum of research stages – basic, transitional, applied – in order to enhance Missouri's position as a national leader in the agricultural sciences. These research projects will focus on both animal and plant science with a particular emphasis on building instrumentation and research equipment capacity, which is a major factor in attracting federal research dollars and, of course, serves as the tool that allows research scientists to develop new technologies. Specific projects will focus on measuring agriculture impacts on Missouri streams, developing livestock odor abatement strategies, improving feed efficiency for pasture-based dairy and beef cattle operations, and extensive study of plant genetics and proteins to improve crop yield. Findings from this research will not only enhance animal and plant productivity, but improve citizens' quality of life while contributing to Missouri economic development efforts.

The Gateway Project consists of nine subprojects organized under three research areas as outlined below. Reports on the progress of each of the subprojects begin on the following page.

Update:

I.A MAINTAINING WATER QUALITY ASSOCIATED WITH ANIMAL & PLANT AGRICULTURE - INFLUENCE OF CONFINED ANIMAL FEEDING OPERATIONS ON BASEFLOW NUTRIENT AND E. COLI CONCENTRATIONS IN NORTHERN MISSOURI STREAMS

Principal Investigator: John R. Jones

In 2008 we selected and ground-checked 95 watersheds and stream sampling sites in Northern Missouri (>39.4° N). Sites were culled from a pool of ~300 watersheds of 30-80 km2 selecting catchments without municipal or industrial point sources, with minimal urban development and representing a broad mix of dominant land-cover types (crop, pasture, forest). Half the selected sites have CAFO's in their watershed or in one or more adjacent catchments. All sites were sampled 4 times during the growing seasons of both 2008 and 2009. Laboratory work was finished in October. Preliminary data analysis suggests little or no effect of CAFO operations on base-flow stream water quality. Final data analyses are currently underway.

I.B MONITORING AND MANAGEMENT OF ANIMAL ODOR - ODOR ABATEMENT/ WATERQUALITY

Principal Investigator: Monty Kerley

Discovery of diet formulations that reduce waste excretion were used to position this research program in concert with efforts to register a process verified program (PVP) with USDA on animal testing and selection programs that reduce greenhouse gas emission (GHG). The importance of this is that the PVP will result in beef producers identifying animals and management strategies that reduce waste excretion. Should cap

and trade legislation pass in the US, beef producers enrolled in or using PVP approved animals will likely be positioned to take advantage of carbon credits issued for steps taken to reduce GHG. Demonstration research has also been done showing that maintaining waste in animals pens at minimum dry matter reduces malodor almost completely. Absorbent material used was sawdust. While this management procedure is simplistic, this is the very reason it can be adopted and remedy odor production from confined beef feeding facilities. Finally equipment used to identify optimum end-point of beef feeding (prevention of prolonged feeding and extra waste production) was first installed at UMC and has led to development of new technologies to measure water intake. Water intake is being studied for its relationship to feed intake, ability to identify sickness in cattle, and potential for metering medicinals to animals.

I.C IMPROVED EFFICIENCIES IN PASTURE-BASED DAIRY OPERATIONS

Principal Investigators: Rob Kallenbach, Richard Crawford

Pasture-based dairy systems in Missouri have grown exponentially over the past 5 years, investing more that $100 million in capital and adding nearly 24,000 dairy cows to the state. While these operations are undeniably successful, one of the most common management issues these producers face is monitoring pasture availability and nutritive value. To date we have evaluated four methodologies for determining dry matter on-offer in pastures. Cool-season grass/legume pastures grazed by dairy cows have been evaluated for pasture dry matter, forage quality, and resultant milk yield every three weeks over the last 2 years. Preliminary results show that the rising plate meter system and the C-DAX equipment function most consistently over a wide range of pasture dry matter yields. Visual appraisal actually had a higher coefficient of determination (R2) than the rising plate meter but the errors, especially at higher levels of forage dry matter were large.

Additionally, as part of this grant we purchased new automated milking equipment for the dairy parlor at the Southwest Center. Installation of this equipment is nearly complete and already gives us the ability to measure milk yield on a daily basis. This addition provides us with the capacity to conduct detailed evaluations of how daily fluctuations in pasture availability and nutritive value impact milk production. To our knowledge, MU will have the only pasture-based dairy operation in the US that can provide information on how variations in pasture change day-to-day milk yields.

I.D GENETIC IMPROVEMENT OF MISSOURI'S BEEF HERD - INCORPORATING SELECTION FOR METABOLIC EFFICIENCY INTO BEEF PRODUCTION-MOVING MITOCHONDRIAL METABOLISM INTO BEEF PERFORMANCE TESTING ENTERPRISES

Principal Investigator: Monty Kerley

Discovery that ratio of complex proteins in mitochondria were predictive of animal efficiency status was used to evaluate high throughput methods of assessing complex protein status between efficient and inefficient animals. Research to date has shown that ratio of complex I activity to complex I concentration, weighted for pyruvate dehydrogenase activity, is predictive of animal efficiency status. These measurements are reduced to analytical kits, allow high throughput capability, and require only a blood sample to supply mitochondrial proteins. Research recently conducted has also demonstrated the importance of selection for efficiency. Cows were phenotyped for efficiency and sires were selected based upon the same criteria. Selecting against the one-third least efficient cows improved progeny feed efficiency 13% and selecting for an efficient sire compared to an inefficient sire improved progeny feed efficiency over 15%. Few technologies can have similar impact to reducing beef cattle production costs.

I.E FEED MILL RENOVATION TO SUPPORT CONTEMPORARY ANIMAL NUTRITION RESEARCH AND MEET THE NEEDS OF THE MISSOURI ANIMAL INDUSTRY

Principal Investigator: Rodney Geisert

The University of Missouri Feed Mill was built in 1963 and continues to produce approximately 2,000 tons of experimental feed per year. This facility provides specialized feed for swine, poultry, beef, dairy, sheep and horses. The experimental diets made by this feed mill are critical to our state's agriculture research. The Division of Animal Sciences feed mill functions to meet the need of delivering multiple experimental diets for testing several animal species at the research farms and centers. Currently, the eight storage bins have been built which gives the Feed Mill a corn storage capacity of approximately 80,000 bushels. We are currently working to get equipment for the Pellet Mill and the legs and auger system for the grain bins installed.

II.A ON-FARM ENERGY PRODUCTION - INTEGRATED SYSTEMS APPROACH TO BIOMASS UTILIZATION

Principal Investigators: Jinglu Tan, Bill Jacoby, Gene Stevens

The project has been divided into three sub-projects: 1. Biomass production (Gene Stevens), 2. Biomass handling and process modeling (Jinglu Tan), and 3. Biomass conversion (Bill Jacoby).

Biomass production: We have been conducting tests to determine the best nitrogen fertilization rate for biofuel production from sweet sorghum. Seven nitrogen rates were applied to sorghum planted three types of soil. Juice samples from stalks were collected and frozen for laboratory sugar testing in the future. The biomass ratio between leaves and stalks was affected by nitrogen rates ($P=0.01$) and soil type ($P=0.0005$). Soil type was significant but N rate was not significant for fresh and dried biomass yields. A significant difference was found among soil types when analyzing the stalk yield ($P<.0001$) and N rates ($P=0.02$). Although sweet sorghum is N efficient, attention needs to be paid to the soil type and N fertilization.
We have been determining the best time to harvest switchgrass to minimize nutrient removal for sustainable production. The maximum biomass yield was obtained in October (21.9 Mg/ha) with slight loss of biomass October to November because of seed fall and wind loss. Nitrogen accumulation in the biomass decreased more than four fold from July to November. The least decrease was observed in micronutrients such as Zn, Cu, Mn, and Fe. November was the optimum time to harvest to maximize the yield while minimizing nutrient removal.

Biomass handling and processing modeling: A logistic model was developed for a biomass utilization system and implemented in ExtendSim. The model allows for system simulation and analysis of biomass supply chain in terms of economic viability and energy balance. The supply 5 chain network was divided into three main subsystems including crop production, biomass handling and logistics, and biomass processing. After validation, the model was used to simulate different conditions and practices so that favorable system configurations and realistic limitations could be determined to maximize net energy output and economic viability. The model was based on the operation of Show Me Energy Coop, a local biomass pelletization plant near Centerview, MO. The simulation results indicated potential benefits from increased truck capacity for transportation, expanded plant capacity, and improved process throughput. The study also suggested that corn stover, a biomass material the plant uses, provides better performance than other biomass materials such as switchgrass and miscanthus.

Biomass conversion process: This sub-project focuses on thermochemical conversion processes. We are exploring oxidative processes (*e.g.* exothermic combustion), reductive processes (*e.g.* endothermic gasification or pyrolysis), extractive processes (*e.g.* soybean oil or sweet sorghum) and synthetic processes (*e.g.* aromatics from lignin),

We are evaluating the extraction of soybean oil using supercritical carbon dioxide (scCO2) relative to the hexane crush process currently used in the soy oil extraction. We are developing a "seed-to-use" process model in ExtendSim. We are working on a second model substituting the scCO2 process for the hexane

crush process. We have been exploring the scCO2 process on both the gram-scale and the kilogram-scale. The results showed that only the particle size had a significant effect (smaller is better). This indicates that the extraction rate can be further optimized and that the expensive de-hulling and flaking processes may not be required when using scCO2 as an extraction solvent. We continue to explore gasification of biomass in supercritical water. We have documented the utility of this technique for a wide variety of biomass. We also gasified sweet sorghum samples grown in four different environments (soil type and N rate). The analysis of variance showed at least one of them has a different vapor yield than the others.

II.B GRAPE GENOMICS AND VITICULTURE - SURVEY AND CHARACTERIZATION OF VIRUS AND VIRUS-LIKE DISEASES IN MISSOURI VINEYARDS

Principal Investigators: Keith Steigler, Wenping Qiu

In addition to collaboration with faculty from Missouri State University to survey vineyards and collect tissue samples for virus analysis, two activities were conducted by personnel from the Institute for Continental Climate Viticulture and Enology in pursuit of the goals of this project, a survey of plant pathogenic nematodes in vineyards in Missouri and Arkansas and a study of the impact of viruses on growth, yield, and fruit composition of 'Chardonel' grapevines.

The nematode survey was conducted in late October and early November after grape harvest was completed. Twenty-two commercial vineyard operations representing 6 grape production regions around Missouri and 8 commercial vineyard operations representing 3 grape producing regions in Arkansas were selected as sites from which soil samples were to be collected for nematode analysis. Seventy-six samples were collected from Missouri vineyards and 31 samples were collected from Arkansas vineyards. These samples represented the most commercially important cultivars in Missouri (14) and Arkansas (15), and further represented several grapevine species of varying susceptibility to virus problems including *Vitis aestivalis*, *V. labruscana*, *V. vinifera* and several multi-species hybrids in both Missouri and Arkansas as well as *Vitis rotundifolia* (the muscadine grape) in Arkansas. The samples were analyzed for the presence of plant pathogenic nematodes and nematodes in each sample were identified as to genus and, in the case of dagger nematodes, species. Of particular interest in this survey was the dagger nematode species *Xiphinema americanum*, a known vector of several viruses affecting grapevines. Nematodes found present in vineyards included *Xiphinema americanum* (dagger), *Paratylenchus* spp. (pin), *Criconemoides* spp. (ring), *Helicotylenchus* sp. (spiral), *Pratylenchus* spp. (root lesion), *Tylenchorenscus* spp. (stunt), *Hemicycliophora* spp. (sheath), *Meloidogyne* spp. (root knot), and *Tylenchulus* spp. (citrus). Of these eight nematode genuses, *Xiphinema*, *Paratylenchus*, *Criconemoides* and *Helicotylenchus* were the most common, being found in 105, 46, 50 and 86 samples, respectively. While there were few consistent trends in nematode occurrence among the *Vitis* species, native American cultivars were found to have root lesion nematode population levels above the economic threshold (ET) and hybrid cultivars had ring nematode population levels above the ET. In view of the growing number of cases of nepovirus-infected vineyards in the region, the presence of *Xiphinema americanum*, a known nepovirus vector, in 105 of the 107 samples is a significant finding and indicates the possibility that the nepovirus problem in regional vineyards may be more widespread than previously thought.

In the second activity conducted as part of this project, two vineyards where vines displayed nepovirus-like symptoms were selected in 2008 to conduct a multi-year study of the impact of virus infection on the performance of 'Chardonel' grapevines, a winegrape cultivar that has been widely impacted by nepoviruses in recent years, showing declining vigor and production. Additionally, in 2009 a vineyard of 'Cabernet Sauvignon' grapevines with vines displaying nepovirus-like symptoms was added to the multi-year study. The studies were conducted in commercial vineyard operations in south-central Missouri and in the Missouri River Valley area of central Missouri ('Chardonel') and a commercial vineyard operation in the

Missouri River Valley area of east-central Missouri ('Cabernet Sauvignon'). At each location, within large blocks of vines, vines were selected for data collection based on the presence or absence of visible virus symptoms and classified as symptomatic or asymptomatic. Tissue samples were collected from each vine for analysis by Dr. Wenping Qiu to confirm the virus-infection rating given to each. At the time of commercial harvest, berry samples were collected from each vine for fruit composition analysis and then each vine was individually harvested with grape cluster counts and yield weights being collected for each vine. The berry samples were analyzed in the ICCVE lab for berry number per cluster, average berry weight, soluble solids (mainly sugar) content, pH and titratable acidity of the juice. The vines will be pruned during the winter and the weight of wood removed from each vine will be recorded to provide a measure of vine growth as affected by virus infection. While observation would indicate that vine growth and yield were reduced in vines with visible signs of infection as compared to asymptomatic vines, this data is still being analyzed and results are not yet available. The selected vines in each of these blocks will be followed for several years to determine the long-term effects of virus-infection on vine health, growth, productivity and fruit composition.

III.A ENHANCE STATEWIDE EQUIPMENT INFRASTRUCTURE SUPPORTING PLANT AND ANIMAL GENOMICS

Principal Investigator: Jeremy Taylor

The goal of this subproject was to acquire an Illumina Genome Analyzer "next-generation" sequencing instrument, recruit a technician to support the varied applications of the instrument and to purchase sufficient preliminary reagents to bring the instrument on-line for use by the Missouri plant and animal genome research community. We were also able to leverage the LSTF grant award to secure a discount on the instrument by simultaneously purchasing an Illumina BeadExpress instrument.

The Taylor laboratory in Animal Sciences utilized the instrument for a 35 plate (3,360 sample) project in the summer to genotype 96 single nucleotide polymorphisms within two genes associated with the tenderness of beef. Duane Keisler in Animal Sciences is preparing to use the carboxyl beads for hormonal assays.

GENOME ANALYZER

Equipment performance:

Total Number of Flowcells Processed: 45

Total Raw Sequence Generated: 215.9 Gb (1 Gb = 1,000 million bases of sequence)

Total Pass Filter Sequence Generated: 129.3 Gb (high-quality reads used for analysis)

Organisms sequenced: Arabidopsis, Bovine, Canine, Drosophila, Human, Mouse, Neurospora crassa, Ovine, Porcine, Soybean

Sequencing applications as a percentage of Flow Cell lanes:

Genomic DNA 34.1%

Small RNA molecules (digital gene expression of small RNAs) 32.3%

Immunoprecipitated chromatin (DNA that interacts with proteins) 16.2%

Messenger RNA (transcribed genes) 37.6%

Number of researchers having utilized instrument: 8

UMC 14 (Animal Sciences, Biochemistry, Biological Sciences, Molecular Microbiology and Immunology, Plant Sciences, Veterinary Biomedical Sciences, Veterinary Pathobiology)

UMKC 1 (School of Biological Sciences)

Outside Academic 4 (Concordia University, University of Wyoming, Medical College of Georgia Cancer Center, Stowers Institute)

Reagent Expenditures:
Current expenditures for GAII Reagents in FY09 are $192,237.56. Start-up reagents were $18,760. These reagents were used for pilot projects by six researchers from Animal Sciences, Plant Sciences, Biological Sciences and Pathology & Anatomical Sciences.

Instrument/Upgrades:
Our current instrument configuration is a GAII with a paired-end sequencing module (PEMx). The GAIIx upgrade was purchased with DNA Core equipment funds and installed in July 2009. The cost of this upgrade was $20,000. Briefly, the upgrade includes a larger reagent cooler to enable uninterrupted 100 bp runs and a redesigned manifold which allows the imaging of 120 tiles per lane (previously 100 tiles per lane). The 20% increase in scanned surface area provides approximately 168 million more reads per flow cell. Typical data outputs for a single flowcell are:

Analysis Description	Approximate Raw Data Output (Gb)/Flow Cell
Single Read (42 bases)	7
Single Read (84 bases)	14
Paired-End Read (2x42 bases)	14
Paired-End Read (2x84 bases)	28

FY2008 Funding Summary – Commercialization

Grant Number	Project Title	Principal Investigator	PI Institution	Grant Category	Funds Awarded
13323	Commercialization of Value-Added Food-Grade Soybean Lines Developed by the University of Missouri and New Generation Functional Food Ingredients and Plant-Made Component for Nutritional Retail Products	Alex Stemme and Dr. Henry Nguyen	Mid-America R&D Foundation	Gateway	$738,281
13324	Commercialization of a Proprietary Bull Fertility Test	Dr. Peter Sutovsky	University of Missouri-Columbia	Animal Health	$400,000
13319	Polyhydroxyalkanoates in Transgenic Oilseeds	Dr. Jan Jaworski	Danforth Plant Science Center	Plant Science	$1,136,719
13320	Animal Waste Phosphorous Management Systems	Gary Clapp and Bill Junk	Institute for Industrial and Applied Life Sciences	Odor Abatement, Water Quality, Bioenergy	$325,000
				TOTAL	**$2,600,000**

FY2008 Commercialization Project Summaries

Project #:	13323-2007
Project Title:	Commercialization of Value-Added Food-Grade Soybean Lines Developed by the University of Missouri and New Generation Functional Food Ingredients and Plant-Made Component for Nutritional Retail Products
Award Amount:	$738,281
Center for Excellence:	Statewide
Lead Investigator:	Alex Stemme, Mid-America Research and Development Foundation, Jefferson City; Dr. Henry T. Nguyen, Director at the National Center for Soybean Biotechnology, University of Missouri-Columbia
Collaborators:	W. J. (Bill) Cook, Missouri Food and Fiber; Ryan Schmidt, Soy Labs, LLC; David A. Sleper, National Center for Soybean Biotechnology; J. Grover Shannon, Delta Research Center, UM; and Richard J. Hofen, University of Missouri-Columbia

Summary:
This project seeks to commercialize several lines of technologically-enhanced soybeans developed by University of Missouri research scientists. These new soybeans have unique and special characteristics such as higher levels of oil and protein, or larger quantities of certain soy peptides demanded by the nutrition industry. These proprietary soybean varieties – grown in Missouri farmers' fields – will then be processed in Missouri into novel "functional food" ingredients (e.g. foods with benefits beyond basic nutrition).

This project will establish Missouri as the nation's leading intersection of plant science research and heart-healthy soy protein and functional food products – escalating the success of the National Center for Soybean Biotechnology at the University of Missouri. The project also assists in creating and developing a new commercial research institution, AgBorn Genetics, LLC. It will also attract and further develop the commercial firm, Soy Labs, LLC. Ultimately, the project will deliver new, Missouri-founded plant science technologies to the global marketplace in the form of functional food ingredients.

Update: This project was successful in helping to recruit Soy Labs to the Missouri Plant Science Research Center in Mexico, Missouri – which is in the final stages of development and implementation – catapulting Missouri as the center of plant science research and the soy in human health marketplace. This project led to the production of soybeans from Missouri fields harvested this fall, which should be then processed in Missouri as soon as equipment is installed and the construction is completed at the new center.

This project will allow the nutraceutical and functional food company, Soy Labs, to grow their Lunasin XP® enriched soybeans from University of Missouri research scientists on Missouri farms and then process those specialty soybean lines (exhibiting powerful cholesterol-lowering properties) at the Missouri Plant Science Research Center in Mexico, Mo. The resulting functional food ingredients will then be marketed to the nutrition industry throughout North America and to international markets from Europe to Southeast Asia.

Screening soybean germplasm for various nutritional compounds including isoflavones, small peptides and total proteins has been performed. This project has identified soybean lines with higher isoflavone components (more than a dozen-fold higher than existing cultivars). In addition, soybean lines with higher levels of oil from this grant project are being commercialized throughout Missouri, the Delta and the Mid-South region of the U.S.

Project #:	13324-2007
Project Title:	Commercialization of a Proprietary Bull Fertility Test
Award Amount:	$400,000
Center for Excellence:	Statewide
Lead Investigator:	Dr. Peter Sutovsky, University of Missouri-Columbia
Collaborators:	Dr. David Patterson, University of Missouri-Columbia

Summary:
This proposal seeks to commercialize a new, patented bull fertility test that will improve reproductive health and performance of dairy and beef bulls, thus adding to the bottom lines of many Missouri dairy and beef cattle producers. Current fertility evaluation in bulls is based on subjective methods introduced in the 1950's. The test that is the subject of this grant represents an accurate, inexpensive, and commercialized viable approach to improving efficiency in this area. This method centers on detection of sperm surface molecules that are found only in defective sperm cells, which provides a quick evaluation of bull fertility and diagnosis of reproductive disorders. The goal of this project is to develop and commercialize the following products for fertility testing in bulls: **AIM 1**) Veterinarian's office bull fertility test kit; **AIM 2**) Reference laboratory service for bull fertility testing; and, **AIM 3**) Nanotechnology based semen purification kit to be used by artificial insemination companies. A start-up company spun off by the University of Missouri, or licensing to a Missouri-based animal health/artificial insemination company will be pursued as avenues for commercialization.

Update: Under **AIM 1**, a simple procedure has been developed for the labeling and evaluation of bull sperm cells attached to a microscopy slide. In brief, a small amount of bull semen is mixed with ice-cold methanol that acts as a fixative, spread on a coated slide and allowed to evaporate, effectively affixing the sperm cells to a microscopy slide. The slide is then overlaid with a fluorescently labeled probe, an antibody that binds to ubiquitin protein found exclusively on the surface of defective sperm cells. The slide is incubated with the antibody for 15 min, washed by submerging in water, covered with a coverslip and observed under epifluorescence microscope. The light microscopic ubiquitin-based sperm evaluation procedure has been tested by veterinarians at a major US artificial insemination (AI) company.

Toward commercialization of a reference laboratory test under **AIM 2**, ubiquitin testing has been coordinated with the development of a testing platform based on a dedicated sperm flow cytometer, recently launched by a major US and worldwide purveyor of supplies and instruments for AI industry and farmers. A large scale trial has been completed on 160 bulls with a goal of validating the ubiquitin test on the sperm flow cytometer platform. Strong negative correlations were found between ubiquitin and conventional parameters of semen quality. Non-disclosure agreements are in place with the Company, and the intent is to make this technology a part of the line-up of pre-set tests offered with the said instrument, which is being marketed in USA and worldwide.

Ubiquitin-binding nanoparticles for the removal of defective sperm cells from bull semen (**AIM 3**) have been developed and tested successfully in bull sperm depletion trials. Measuring sperm viability by flow cytometry demonstrated a statistically significant improvement in the viability (percentage of live/viable sperm cells) in semen samples depleted by our nanoparticles. Enrichment of defective sperm cells has been documented in the nanoparticle fraction discarded after depletion. This functional prototype is a result of collaboration with a US-based biotech company specializing in magnetic purification of cultured cells. The intent is to market this product directly to end users in AI industry, or to offer the semen depletion kit through cooperation with a major US AI-industry supplier. A secondary market is being explored for the use in human infertility clinics.

Project #:	13319-2007
Project Title:	Polyhydroxyalkanoates in Transgenic Oilseeds
Award Amount:	$1,136,719
Center for Excellence:	St. Louis
Lead Investigator:	Dr. Jan Jaworski, Donald Danforth Plant Science Center
Collaborators:	Edgar Cahoon and Joseph Jez, Donald Danforth Plant Science Center

Summary:
Polyhydroxyalkanoates (PHA) are polyesters that are produced by microbes. Their physical properties and environmental benefits are uniquely suitable for industrial and medical use as biobased, sustainable and biodegradable plastics. This project is collaborative between scientists at the Donald Danforth Plant Science Center and Metabolix Inc. based in Cambridge, MA. Metabolix is a winner of the "Presidential Green Chemistry Award" for the development of PHA in plants, the focus of this commercialization project. The work funded by this grant will develop the technological foundation for efficiently producing these biomaterials in non-food crops and Metabolix scientists will be located at the Nidus Center for Scientific Enterprise in St. Louis. The project objectives are to produce bioplastics in transgenic oilseeds as a value added feedstock for biodiesel biorefineries.

In the first year of the project the Donald Danforth Plant Science Center and their oilseed experts has established a strategic research and commercialization collaboration with Metabolix, a bioscience company, for the production of the biodegradable plastic, polyhydroxyalkanoate (PHA), in plants. They are collaborating on a project to develop an advanced industrial oilseed crop to produce bioplastics.

The proposed work is to develop the technological foundation for efficiently producing these biomaterials in non-food crops Brassica juncea (Indian mustard) and Camelina sativa (False flax) with seed-specific expression of genes for the production of PHA. Metabolix has established a new group at the Nidus Center in St. Louis that presently consists of 2 Ph.D. scientists and a laboratory technician. It is anticipated that the size of this group will continue to grow. The goal is to have plants with seeds containing 5% dry weight PHA within 3 years and reduce to practice the production of a PHA-based plastic within 5 years.

Most of research in year one was carried out with Camelina sativa because of its short 3-month growth cycle. They have succeeded in producing numerous transgenic Camelina plants that synthesize significant levels of PHA. PHA levels of greater than 5% were achieved, but these high levels were toxic to the seed. They have revised their strategy to include the use a "gene switch" that will allow them to produce plants that only produce PHA when treated with the chemical methoxyfenozide. Preliminary results indicate that this strategy is working very well it will allow them to grow some plants with little or no PHA to yield viable seeds and grow other treated plants to produce seeds synthesizing high levels of PHA. Initial findings suggest that they will exceed their target of 5% PHA.

Update: This project was very successful in meeting objectives. In the course of the research, numerous lines of transgenic camelina plants were generated that produce varying levels of PHB, the biodegradable plastic that is the target of this study.

Project #:	13320-2007
Project Title:	Animal Waste Phosphorous Management Systems
Award Amount:	$325,000
Center for Excellence:	Kansas City
Lead Investigator:	Dr. Gary Clapp, Institute for Industrial and Applied Life Sciences and Bill Junk, DT Search & Designs, LLC
Collaborators:	Dean Thompson, DT Search & Designs, LLC and Gina Becker, Advanced Manufacturing Institute; Sigifredo Castro, Advanced Manufacturing Institute; Bret Lanz, Advanced Manufacturing Institute; Kylo Heller, KLA Environmental Services, Inc;.Frank Mercurio, KLA Environmental Services, Inc.; and Rick McKee, Kansas Environmental Management Associates

Summary:
The EPA (Environmental Protection Agency) regulations require CAFO's to balance the waste nutrients they apply to crop land. Typically phosphorous is the most limiting factor. The recent surge in ethanol production has increased the amount of distiller's grain (an ethanol byproduct) available for use as a feed ingredient. DDGs (Dried Distiller's Grains) have been shown to increase the amount of phosphorous in animal waste by as much as 120 percent. This, coupled with the EPA waste application requirements, will require feedlots and dairies to either acquire more land or greatly reduce the use of DDGs. In most cases, additional land is unavailable. Therefore, the only alternative is to decrease the phosphorus concentration in the waste, thereby allowing the CAFO to comply with EPA regulations and expand the use of DDGs.

This project will demonstrate an economical, user-friendly system that significantly lowers phosphorous concentrations in lagoon wastewater. Funds will be used to construct a farm-scale, pilot phosphate reduction system for lagoon wastewater on Concentrated Animal Feeding Operations (CAFO's) that smaller-scale versions show reduce phosphorus levels by at least 75 percent.

The Institute for Industrial and Applied Life Sciences, has continued its progress on production of a viable commercial scale phosphorous reduction system that would be used to treat the liquid wastes at CAFOs. The information gathered during the pilot lab scale and small farm scale operations were put to use and allowed for the build of a commercial system.

The commercial system was constructed and assembled on a CAFO that could hold 60,000 head of cattle. Construction delivery and assembly took place during the spring and early summer of 2008. The initial assembly went relatively smooth; however, the early summer did not bring enough rain to wash the animal waste from the CAFO holding pens in to the storage lagoons. Therefore, they were forced to wait for proper weather conditions. The rains finally arrived at their CAFO in mid-August and they were able to initiate their shake down evaluation runs.

The performance runs from August and September were showing very good commercialization success by removing greater than 50% of the phosphorous in a single pass. However, the October cool weather seemed to reduce the performance of the commercial unit to 30% reduction. This seasonal fluctuation is to

be expected as the chemistry of the lagoons with the seasons, as well as the performance of the waste management system.

They have been constantly monitoring the lagoon chemistry and they are continuing their shake-down runs this month as the cold rainy season begins. The pH, Total Phosphorous, Phosphate, Calcium, and Magnesium are all contributors to the performance of the reduction runs. They believe they understand the low Phosphorous reduction performance from October and November and have addressed this by adding a mixing chamber for the ammonia and the water prior to entering the reactor. They process looks to perform much better with adequate mixing and when higher temperatures are employed.

The system has performed quite well under some pretty realistic and difficult conditions. These conditions would actually represent the real world challenges faced by the end user. As a result, they have initiated drawings for the unit that will be sold as a commercial treatment. A local St. Joseph manufacturer is very interested in building these units once they have worked out the operational challenges associated with a full year. Functional tests will continue the next few months and changes will be made if needed.

Update: New EPA (Environmental Protection Agency) regulations that require CAFO's to balance the waste nutrients they apply to crop land went into effect in February 2009. Typically phosphorous is the most limiting factor. The recent surge in ethanol production has increased the amount of distiller's grain (an ethanol byproduct) available for use as a feed ingredient. DDGs (Dried Distiller's Grains) have been shown to increase the amount of phosphorous in animal waste by as much as 120 percent. This, coupled with the EPA waste application requirements, will require feedlots and dairies to either acquire more land or greatly reduce the use of DDGs. In most cases, additional land is unavailable. Therefore, the only alternative is to decrease the phosphorus concentration in the waste, thereby allowing the CAFO to comply with EPA regulations and expand the use of DDGs. This project resulted in the development of an economical, user-friendly system that significantly lowers phosphorous concentrations in lagoon wastewater. Funds were used to construct a farm-scale, pilot phosphate reduction system for lagoon wastewater on Concentrated Animal Feeding Operations (CAFO's).

Activity this past year has centered on addressing the inconsistency and low levels of P being removed from the lagoon. Several modifications to the Farm Scale Unit (Phred) were studied and it was determined that injecting ammonia gas while maintaining adequate ammonia pressure under all temperature conditions was the best solution. The modifications to accomplish this were implemented and made a permanent part of Phred. It was also determined that the performance of each individual Phred unit would vary depending on the environmental conditions at the specific CAFO. Therefore all proposals and resulting programs will be individualized.

Phred has been approved for inclusion on the USDA NRCS Environmental Quality Improvement (EQIP) list. Foe EQIP eligible CAFOs the USDA will pay up to $187,500 toward the purchase price of Phred. Sales of Phred officially stared in December 2009 and presently 1 CAFO has made application for EQIP funding to purchase a unit.

COMMUNICATION OF AUDIT RESULTS

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

June 30, 2009

December 7, 2009

To the Board of Directors of
Missouri Life Sciences Research Trust Fund

We have audited the financial statements of Missouri Life Sciences Research Trust Fund for the year ended June 30, 2009, and have issued our report thereon dated December 7, 2009. Professional standards require that we provide you with the following information related to our audit.

Our Responsibility under U.S. Generally Accepted Auditing Standards

As stated in our engagement letter dated November 5, 2009 our responsibility, as described by professional standards, is to express an opinion about whether the financial statements prepared by management with your oversight are fairly presented, in all material respects, in conformity with the basis of cash receipts and disbursements, which is a comprehensive basis of accounting other than accounting principles generally accepted in the United States of America. Our responsibility is to plan and perform the audit to obtain reasonable, but not absolute, assurance that the financial statements are free of material misstatement. Our audit of the financial statements does not relieve you or management of your responsibilities.

As part of our audit, we considered the internal control of Missouri Life Sciences Research Trust Fund. Such considerations were solely for the purpose of determining our audit procedures and not to provide any assurance concerning such internal control. Our internal control findings and other recommendations are included in a separate communication of significant deficiencies letter.

Significant Accounting Policies

Management is responsible for the selection and use of appropriate accounting policies. In accordance with the terms of our engagement letter, we will advise management about the appropriateness of accounting policies and their application. The significant accounting policies used by Missouri Life Sciences Research Trust Fund are described in Note 1 to the financial statements. No new accounting policies were adopted and the application of existing policies was not changed during the fiscal year. We noted no transactions entered into by the Fund during the year for which there is a lack of authoritative guidance or consensus.

Audit Adjustments

For the purposes for this letter, professional standards define an audit adjustment as a proposed correction of the financial statements that, in our judgment, may not have been detected except through our auditing procedures. An audit adjustment may or may not indicate matters that could have a significant effect on the Fund's financial reporting process (that is, cause future financial statements to be materially misstated).

We did not propose any audit adjustments for the year.

Accounting Estimates

Accounting estimates are an integral part of the financial statements prepared by management and are based on management's knowledge and experience about past and current events and assumptions about future events. Certain accounting estimates are particularly sensitive because of their significance to the financial statements and because of the possibility that future events affecting them may differ significantly from those expected.

Difficulties Encountered in Performing the Audit

We encountered no significant difficulties in dealing with management in performing and completing our audit. All of the Fund's personnel cooperated with us fully during our audit.

Disagreements with Management

For purposes of this letter, professional standards define a disagreement with management as a financial accounting, reporting, or auditing matter, whether or not resolved to our satisfaction, that could be significant to the financial statements or the auditor's report. We are pleased to report that no such disagreements arose during the course of our audit.

Management Representations

We have requested certain representations from management that are included in the management representation letter dated December 7, 2009.

Management Consultations with Other Independent Accountants

In some cases, management may decide to consult with other accountants about auditing and accounting matters, similar to obtaining a "second opinion" on certain situations. If a consultation involves application of an accounting principle to the Fund's financial statements or a determination of the type of auditor's opinion that may be expressed on those statements, our professional standards require the consulting accountant to check with us to determine that the consultant has all the relevant facts. To our knowledge, there were no such consultations with other accountants.

Other Audit Findings or Issues

We generally discuss a variety of matters, including the application of accounting principles and auditing standards, with management each year prior to retention as the Fund's auditors. However, these discussions occurred in the normal course of our professional relationship and our responses were not a condition to our retention.

We wish to thank the Missouri Life Sciences Research Trust Fund personnel for their assistance during the course of our audit. We will be pleased to discuss these or any other matters at your convenience. This information is intended solely for the use of Board of Directors and management of Missouri Life Sciences Research Trust Fund and is not intended to be and should not be used by anyone other than these specified parties.

Very truly yours,

Graves and Associates, CPAs, LLC

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

Jefferson City, Missouri

INDEPENDENT AUDITORS' REPORT

For the Year Ended June 30, 2009

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INDEPENDENT AUDITORS' REPORT

To the Board of Directors of
Missouri Life Sciences Research Trust Fund:

We have audited the accompanying financial statements of the **Missouri Life Sciences Research Trust Fund** (a component unit of the State of Missouri), as of and for the year ended June 30, 2009, which collectively comprise the Fund's basic financial statements as listed in the table of contents. These basic financial statements are the responsibility of the Fund's management. Our responsibility is to express an opinion on these financial statements based on our audit.

We conducted our audit in accordance with auditing standards generally accepted in the United States of America. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and the significant estimates made by management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.

As discussed in Note 1, the Fund prepares its financial statement on the basis of cash receipts and disbursements, which is a comprehensive basis of accounting other than accounting principles generally accepted in the United States of America.

In our opinion, the financial statements referred to above present fairly, in all material respects, the respective cash basis financial position of the fiduciary activities of the **Missouri Life Sciences Research Trust Fund** as of June 30, 2009, and the respective changes in cash basis financial position thereof for the year then ended in conformity with the basis of accounting described in Note 1.

Management has elected to omit the Management's Discussion and Analysis section from the audit report.

GRAVES AND ASSOCIATES, CPAs, LLC
Jefferson City, Missouri

December 7, 2009

BASIC FINANCIAL STATEMENTS

The basic financial statements include integrated sets of financial Statements as required by the GASB. The sets of statements include:

- Fund financial statements
  - Fiduciary Fund

In addition, the notes to the financial statements are included to provide information essential to a user's understanding of the basic financial statements.

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

NOTES TO THE FINANCIAL STATEMENTS

FOR THE YEAR ENDED JUNE 30, 2009

NOTE 1 – NATURE OF OPERATIONS AND SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES:

The accompanying financial statements include the transactions of all funds under the management and control of the Fund's Board. The Fund is included as a component unit of the State of Missouri.

The Missouri Life Sciences Research Trust Fund (the Fund) was established by the Missouri General Assembly beginning in fiscal year 2007 and in perpetuity thereafter. Moneys in the Fund shall be used strategically, in cooperation with other governmental and not-for-profit entities, to enhance the capacity of the State of Missouri's ability to perform research to better serve the health and welfare of the residents of the State of Missouri as a center of life sciences research and development by building on the success of research institutions located in Missouri, creating in and attracting to Missouri new research and development institutions, commercializing the life sciences technologies developed by such institutions, and enhancing their capacity to carry out their respective missions. Its governing body consists of seven members who have general familiarity with the life sciences and current research trends and are appointed by the Governor with the advice and consent of the Senate.

The Fund may establish and is to oversee each "center for excellence for life sciences research," which may be located in the St. Louis and Kansas City areas and Springfield in addition to a Missouri Statewide center.

The Fund is to be held separate and apart from all other public moneys and funds of the State, including but not limited to the tobacco securitization settlement trust fund. The state treasurer shall deposit into the fund twenty-five percent of all moneys received from the master settlement agreement.

No more than ten percent of the moneys shall be used for the construction of physical facilities and further provided that in any fiscal year eighty percent of the moneys shall be appropriated to build research capacity at public and private not-for-profit institutions. Twenty percent of the moneys shall be appropriated for grants to public or private not-for-profit institutions to promote life science technology transfer and technology commercialization. Of the moneys appropriated to build research capacity, twenty percent shall be appropriated to promote the development of research of tobacco-related illnesses.

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

NOTES TO THE FINANCIAL STATEMENTS

FOR THE YEAR ENDED JUNE 30, 2009

NOTE 1 – NATURE OF OPERATIONS AND SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES: (Continued)

A. Financial Reporting Entity

The Fund's financial reporting entity is comprised of the following:

Primary Government: Missouri Life Sciences Research Trust Fund

In determining the financial reporting entity, the Fund complies with the provisions of Governmental Accounting Standards Board Statement No. 14, *The Financial Reporting Entity*, as amended by GASB 39 *Determining Whether Certain Organizations Are Component Units*

B. Basis of Presentation

Government-Wide Financial Statements

The Statement of Net Assets and Statement of Activities display information about the reporting government as a whole. They include all funds of the reporting entity except for fiduciary funds. The statements distinguish between governmental and business-type activities. Governmental activities generally are financed through taxes, intergovernmental revenues and other non-exchange revenues. Business-type activities are financed in whole or in part by fees charged to external parties for goods or services. The Fund does not have any governmental or business-type activities. Therefore, no government-wide financial statements are included.

Fund Financial Statements

Fund financial statements of the reporting entity are organized into funds, each of which is considered to be a separate accounting entity. Each fund is accounted for by providing a separate set of self-balancing accounts which constitute its assets, liabilities, fund equity, receipts, and disbursements. The fund is organized into one major category, fiduciary. The Fund presently has no governmental or proprietary funds. An emphasis is placed on major funds within the governmental categories. A fund is considered major if it is the primary operating fund of the Trust or meets the following criteria:

- Total assets, liabilities, receipts, or expenditures of that individual governmental fund are at least 10 percent of the corresponding total for all funds of that category or type, and
- Total assets, liabilities, receipts, or expenditures of the individual governmental fund are at least 5 percent of the corresponding total for all governmental funds combined.

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

NOTES TO THE FINANCIAL STATEMENTS

FOR THE YEAR ENDED JUNE 30, 2009

NOTE 1 – NATURE OF OPERATIONS AND SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES: (Cont'd.)

B. Basis of Presentation (Cont'd.)

Fund Financial Statements (Cont'd.)

The funds of the financial reporting entity are described below:

Fiduciary Fund

Private Purpose Trust Fund - Fiduciary funds are used to account for resources held for the benefit of parties outside the government.

C. Measurement Focus and Basis of Accounting

Measurement focus is a term used to describe "how" transactions are recorded within the various financial statements. Basis of accounting refers to "when" transactions are recorded regardless of the measurement focus applied.

Measurement Focus

In the fund financial statements, the "current financial resources" measurement focus or the "economic resources measurement focus," as applied to the cash basis of accounting, is used as appropriate:

All governmental funds utilize a "current financial resources" measurement focus. Only current financial assets and liabilities are generally included on their balance sheets. Their operating statements present sources and uses of available spendable financial resources at the end of the period.

Basis of Accounting

In the fund financial statements, fiduciary activities are presented using a cash basis of accounting. This basis recognizes assets, liabilities, net assets, receipts and expenditures when they result from cash transactions. This basis is a comprehensive basis of accounting other than accounting principles generally accepted in the United States of America.

As a result of the use of the cash basis of accounting, certain assets and their related receipts (such as accounts receivable and revenue for billed or provided services not yet collected) and certain liabilities and their related expenses (such as accounts payable and expenses for goods or services received but not yet paid) *are not recorded* in these financial statements.

MISSOURI LIFE SCIENCES RESEARCH TRUST FUND

NOTES TO THE FINANCIAL STATEMENTS

FOR THE YEAR ENDED JUNE 30, 2009

NOTE 1 – NATURE OF OPERATIONS AND SUMMARY OF SIGNIFICANT ACCOUNTING POLICIES: (Cont'd.)

D. Assets, Liabilities and Equity

Cash and Cash Equivalents

For the purpose of financial reporting, "cash and cash equivalents" includes all demand and savings accounts, and certificates of deposit or short-term investments with original maturity of three-months or less.

Equity Classification

It is the Fund's policy to first use restricted net assets prior to the use of unrestricted net assets when an expense is incurred for purposes for which both restricted and unrestricted net assets are available.

Fund Financial Statements:

Fiduciary fund equity is classified as ending net assets.

NOTE 2 – DEPOSITS, INVESTMENTS AND INVESTMENT INCOME:

Deposits

Cash and cash equivalents are invested by the State as part of the State's cash pool. All deposit and investment risk is controlled by the State. Information concerning the State's deposit and investment risks may be found in the State's 2009 Comprehensive Annual Financial Report.

NOTE 3 – RISK MANAGEMENT:

The Fund is exposed to various risks of loss related to torts; theft of, damage to and destruction of assets; business interruptions; errors and omissions; natural disasters; employee injuries and illnesses; and employee health and accident benefits. Commercial insurance coverage is purchased for claims arising from such matters other than employee health benefits. Settled claims have not exceeded this commercial coverage in any of the three preceding years.

NOTE 4 - CONTINGENCIES:

Litigation - Various claims and lawsuits are possible against the Fund. In the opinion of Fund management, the potential loss on all claims and lawsuits will not be significant to the Fund's financial statements.